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BRUCELLA ABORTUS IN THE HORSE.

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THE etiology of the conditions known as fistulous withers and poll evil has for many years been the cause of much controversy. Harness wounds and other injuries have frequently been held responsible, although certain authorities (Williams, 1908; Hieronymi and Gilde, 1934) have endeavoured to prove that traumata were unimportant in the cause of true fistulous withers and poll evil. Pyogenic organisms have also been incriminated. In addition, studies of the abnormalities of the ligamentum nuchæ and associated tissues of the horse have shown that the filarid *Onchocerca cervicalis*, is commonly associated with these conditions. This worm is, however, not infrequently found in the necks of apparently normal horses (Steward, 1933). Bacteriological examinations of fluid from the abscesses have also demonstrated that *Br. abortus* alone or accompanied by other organisms is often present in such lesions. This organism has also been shown to be associated with abortion (Hermann, 1934) and mastitis (Gardinazzi, 1936) in mares and with cases of hock (Bülow, 1936) and pastern (Ebner, 1935) lameness in horses.

As indicated in a report (Duff, 1933) recording the isolation of *Br. abortus* from a case of fistulous withers at Windsor in 1932, further work was undertaken at this laboratory with a view to ascertaining with what frequency the organism was to be found associated with suppurative conditions in horses in Great Britain. Veterinary surgeons were requested to forward samples of material from unopened abscesses met with in the course of their practices. During the years 1933, 1934 and 1935, samples of blood and abscess contents from 85 horses showing unruptured lesions (1st Series) and 91 samples of blood from horses showing ruptured lesions were received (2nd Series).

1st Series.

Bacteriological examination, by direct culture and guinea-pig inoculation, of the samples of abscess contents revealed that 68 or approximately 80 per cent. contained *Br. abortus*. Of the 85 samples, 73 were from fistulous withers, 8 from poll evil, and 4 from horses suffering from both conditions ; 60 (or 82 per cent.) of the withers samples, 4 of the poll samples and all of those from cases of both fistulous withers and poll evil harboured *Br. abortus*.

The agglutination titres of the abscess fluid from which *Br. abortus* was isolated were, one 1 in 20 ; one 1 in 40 ; six 1 in 80 ; sixteen 1 in 160 ; sixteen 1 in 320 ; seventeen 1 in 640 ; and ten 1 in 1,280. Of the cases from which the organism was *not* recovered, two showed no agglutination, two reacted at 1 in 10 ; and two at 1 in 20. The remainder gave titres varying from 1 in 40 to 1 in 1,280. Of all the cases from which both abscess contents and blood were received, the titre of the blood exceeded that of the fluid from the lesions in only four instances.

Particulars of 80 horses from which abscess material was obtained showed 56 draught or farm horses, 13 cobs, 3 Suffolks, 3 hunters, 4 ponies, and 1 thoroughbred. Older animals appear to be more frequently affected, as, while only nine horses were under 5 years of age and 29 were between 5 and 10 years, 42 were over 10 years old. The susceptibility of mares and geldings seems to be about equal, there being 44 and 36 respectively in this series.

Contact with cattle appears to be most significant as, of the 80 horses concerning which reports were received, 74 (or 92 per cent.) had been in contact with cattle, three had not been in contact, and in three cases it was not known if contact had taken place.

Cattle were infected with contagious abortion (Brucellosis) on farms on which 56 of the affected horses were or had been grazing, and not infected on farms on which eight of the horses were kept. In 16 instances it was unknown if the disease was present or not.

2nd Series.

In this series (91) samples of blood were examined from 70 horses affected with fistulous withers, 19 with poll evil and 2 suffering from both conditions. Agglutination tests disclosed that eight samples were negative, 6 reacted at 1 in 20 ; 15 at 1 in 40 ; 18 at 1 in 80 ; 14 at 1 in 160 ; 20 at 1 in 320 ; 6 at 1 in 640 ; and 3 at 1 in 1,280. One sample was hæmolysed when received. It will be observed from these figures that 75 per cent. of the sera examined gave titres of between 1 in 40 and 1 in 320. There appeared to be no relationship between the blood titres and the age of the lesions as indicated by American workers (Frost and Errington, 1935).

As in the previous series, farm or draught horses were very much in the majority, numbering 67, as compared with 4 cobs, 2 Suffolks, 8 hunters, 3 ponies and 2 thoroughbreds. The age factor is again in evidence, as while only 14 horses were 5 years or under,

33 were between the ages of 5 and 10 years, and 39 were over 10 years old. The number of mares and geldings was 48 and 42 respectively.

The apparent significance of contact with cattle is quite as evident in this series as in the previous one. Of the 84 cases reported on by the veterinary surgeons concerned, 75 (or 87 per cent.) had been grazing or housed with cattle, two had not been in contact, two had not been in contact for six months, and in seven cases it was unknown if contact with cattle had taken place.

Cattle were infected with contagious abortion (Brucellosis) on the farms on which 55 of the horses were or had been grazing, and stated not to be infected on the farms on which 15 of the horses were kept. In 14 instances it was not known if the disease was present or not.

In this connection, it is of interest to note that in one instance in America (White and Swett, 1935) horses suffering from fistulous withers appeared to have been responsible for an outbreak of contagious abortion (Brucellosis) in previously non-infected cattle on the same farm.

Vaccine Treatment.

As a result of the number of cases from which it was possible to isolate *Br. abortus* during the first few months of 1933, it was decided to try the effects of a dead vaccine prepared from strains of *Br. abortus* recovered from cases of fistulous withers and poll evil. The vaccine was prepared by growing the organisms on Roux flasks containing Liver Infusion Agar (P.H. 7·2) for four to six days and washing the growth from the surface of the medium with normal saline solution containing 0·3 per cent. phenol. The suspension was standardised to No. 10 tube of McFarland's nephelometer.

Inoculation experiments were carried out on two laboratory horses which were negative for abortus agglutinins, and it was found that with the exception of a small, painless swelling about the size of a walnut, no reaction occurred to one inoculation of 10 c.c.

Reports on the vaccination of 134 horses suffering from fistulous withers and poll evil were received from co-operating veterinary practitioners. Of these 95 (77 fistulous withers, 15 poll evil and 3 with both conditions) recovered, 35 having also been operated upon; 13 showed improvement; 14 showed no improvement; 7 died or were destroyed and 5 died from other causes. The number of cases operated upon is approximate as in some instances the swellings were simply incised to facilitate drainage, whilst in others the radical operation was performed and tissue removed.

The simple, uncontaminated lesions appeared to respond fairly readily to two or three inoculations each of 10 c.c. given subcutaneously low down in the neck (with an interval of 10 days between the injections) while the cases in which pyogenic organisms were present or in which there were pieces of necrotic tissue, were very resistant.

In the latter cases it was found that the aspiration of abscess

contents or the removal of diseased tissue in conjunction with the vaccine treatment assisted the healing process. Recurrences after primary healing occasionally developed, the explanation for which would appear to be that deep-seated pockets of fluid had been forced to the surface by work or exercise. The reopened lesions responded readily to further inoculations of vaccine.

The agglutination titre of the blood seems to give little indication of the results to be expected from the vaccine inoculations, as the abscesses in many cases healed just as rapidly in horses showing a titre of 1/10 to 1/40 as they died in those animals showing high blood agglutination reactions.

A disagreeable feature of the vaccine is that it produces a fairly severe local reaction in *infected* horses. This would seem to be in the nature of an allergic reaction, as in cases in which the vaccine was inoculated into horses showing no abortus agglutinins the reactions were negligible. The sensitivity of the skin of infected horses to injections (subcutaneous or intradermal) of killed cultures of this organism had been claimed to be a reliable guide in diagnosing *Br. abortus* infection (Rossi and Saunié, 1934; Hieronymi and Gilde, 1934).

The local disturbance may take the form of a slough at the site of inoculation; when this occurs there may also be a rise in temperature and loss of appetite.

Bacteriology.

The character of the material from the lesions in which *Br. abortus* was found to be present is fairly typical, being a yellowish-brown, sometimes blood-tinged, glutinous or serous fluid containing whitish yellow or reddish shreds, flakes or lumps of fluffy coagula. These coagula have been aptly likened by certain observers to grains of boiled rice or barley.

Br. abortus was, however, in one instance isolated (by guinea-pig inoculation) from a piece of adventitious tissue, from a withers lesion, forwarded for examination for worm (*O. cervicalis*) infestation.

The system adopted in carrying out the bacteriological examinations of the material received was to sow culture tubes containing agar, liver infusion agar, blood agar and serum agar and to incubate (37° C.) a certain number of each type aerobically, anærobically and in an atmosphere of 10 per cent. CO_2. Four guinea-pigs were also inoculated from each sample of abscess contents. The results of the examination of material from 85 cases were that, *Br. abortus* was isolated in pure culture from 51, nine of which grew ærobically; it was found in conjunction with other organisms in eleven instances; not found in direct culture but recovered from guinea-pig spleen culture in six. Other organisms were found in twelve cases in which examination for *Br. abortus* was negative; and in five instances no growth of organisms of any kind occurred. The length of time during which seeded culture tubes are incubated is most important,

since, although the majority of recovered strains showed visible signs of growth between 3 and 8 days, some strains were incubated for between 11 and 17 days before colonies appeared.

Of the nine strains which grew ærobically, five appeared between 4 and 8 days and four between 12 and 14 days. Growth appeared at the same time as that in CO_2 with three, and 5 to 8 days later than that in CO_2 with six. It has been suggested, when *Br. abortus* grows ærobically in primary culture, that the strain is of " live vaccine " origin. Of the nine cases described above, only two occurred on farms on which the cattle had received inoculations of live vaccine. In this connection it is of interest to mention that *Br. abortus* was isolated ærobically in this country before live vaccine immunisation was commenced (Rpt. of the Deptmtal. Comm. Part 1. 1905).

Unfortunately, as normal routine duties were being carried out during this investigation, time was not available in which to conduct a systematic examination for the presence of *Br. abortus* in the blood samples from reacting horses.

Liver infusion agar (P.H. 7·2) proved to be the most suitable medium for primary growth, although in a number of instances the rate of growth appeared to be equal on all media.

The appearance of the colonies on the original cultures was characteristic, being delicate greenish or amber-coloured and dewdrop-like. The colonies, as a rule, did not tend to coalesce.

A point of some importance in the seeding of culture tubes and in the inoculation of guinea-pigs is that the flakes or lumps of coagula found in the abscess contents should be broken up, as the organism appears to be more readily isolated thereafter than when the fluid alone is used. The fact that this precaution was not always taken in the earlier stages of the investigation may explain the failure at that time to recover the organism from a number of the samples of material examined.

Agglutination tests of the specimens of abscess fluid from which *Br. abortus* was isolated revealed that in two cases the titre was 1 in 20 and 1 in 40. This titre is much lower than that generally accepted (Hieronymi and Gilde, 1934 ; Freund, 1935 ; Gilde, 1935 ; Schellner, 1934 ; Hermann, 1934 ; Hieronymi, 1936 ; Lütje, 1936) as indicating infection.

The age of the lesions harbouring the organism is of considerable interest. In one case the visible lesion was reported to be under 1 week old ; in 26 cases between 1 week and 1 month ; in 18 cases between 1 and 3 months ; in 14 cases between 3 and 6 months ; in three cases between 6 months and 1 year, and in two cases over 1 year. In four cases the age of the lesion was unknown.

Other organisms found in the samples of abscess contents in conjunction with *Br. abortus* (eleven cases) and when *Br. abortus* appeared to be absent (twelve cases) were streptococci, staphylococci and moulds. None of the streptococci were hæmolytic and the other organisms were apparently non-pathogenic to guinea-pigs. It was

difficult in the prevailing circumstances to decide whether these organisms had actually been present in the lesions or whether they had gained access to the sample of material as contaminants. The method used for obtaining the samples was to forward by post to a veterinary surgeon, who had previously reported the existence of an unopened case of fistulous withers or poll evil on the farm of a client, a sterilised syringe, with a needle attached by a piece of rubber tubing. After the withdrawal of fluid from a lesion a steel spring clip was placed on the rubber tubing to prevent leakage and the syringe was returned to the laboratory by post. Under these circumstances there was some considerable risk of contamination by organisms from outside sources.

Contrary to results obtained in Russia (Vichelessky and Bobileva, 1935) direct culture would seem to be a more reliable means of isolating *Br. abortus* than guinea-pig inoculation, since in 19 instances the organism was recovered in direct culture when guinea-pig spleen culture was negative, while in only six cases was the guinea-pig spleen culture positive when direct culture showed no growth of *Br. abortus.*

It was the practice to carry out agglutination tests with blood obtained by heart puncture 6 weeks after the inoculation of (four) guinea-pigs with the suspected material. The titres of those animals from which Brucella was subsequently isolated in spleen culture varied between 1 in 80 and 1 in 5,120 ; however, on five occasions it was possible to recover the organism from the spleens of guinea-pigs the sera of which failed to show any agglutinins. On the other hand, two guinea-pigs which had been inoculated with different samples of material and which gave titres of 1 in 160 and 1 in 1,280 did not yield *Br. abortus* on spleen culture although the organism had already been isolated in direct culture from each sample. The other guinea-pigs in the series to which these two belonged all gave negative results in agglutination tests and in spleen culture.

Nodule formation in the guinea-pig spleen does not appear to be a characteristic of the strains of Brucella found in the horse, as in only a few instances was slight nodule formation observed, while in the large majority of cases the spleen was apparently normal or slightly enlarged.

The identity of the organism recovered from each specimen of material was confirmed by antigen tests against known positive and negative bovine sera, by guinea-pig serum tests against four stock *Br. abortus* antigens, by fermentation tests and by potato culture. Neither acid nor gas was produced in glucose, lactose, dulcite, maltose, mannite, saccharose, sorbite, inuline, lævulose, raffinose, arabinose, rhamnose, adonite, galactose, xylose, dextrine, inosite, or salicine. No indol was produced. Litmus milk showed slight alkalinity in 4 days and distinct alkalinity in 9 to 12 days. Potato cultures developed the chatacteristic honey or pale chocolate coloration in 17 days.

Discussion.

It is evident from the information collected in this investigation that *Br. abortus* is present in a very large proportion of cases of fistulous withers and poll evil, and details supplied by co-operating veterinary surgeons seem to indicate that many of the lesions in these conditions result from infection by *Br. abortus* of the atlantal and supraspinous bursæ.

Those cases in which fibrous tissue and necrotic areas are present (and which do not respond to vaccine treatment) probably result from the inflammatory processes which follow the invasion of the parts by pyogenic organisms. The calcified areas which commonly develop after infestation with *O. cervicalis* (Steward, 1933) may also constitute predilection seats for bacterial invasion.

No experiments were undertaken with a view to setting up fistulous withers or poll evil artificially but the results recorded by other workers (Schellner, 1934; Panisset, 1935; Velu, 1935) show that although abscess formation and fibrous tissue development frequently followed the injection of live organisms, alone or along with other substances, all such attempts failed to produce typical lesions.

Summary.

1. 80 per cent. of the material from unopened lesions, varying in age from under 1 week to over 1 year, contained *Br. abortus*.

2. 92 per cent. of the horses in the first series and 87 per cent. of the horses in the second series had been in contact with cattle.

3. 71 per cent. of the treated cases were reported to have recovered as a result of inoculation with a *Br. abortus* dead vaccine, with or without operation.

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He is also much indebted to the 71 veterinary surgeons who gave their active co-operation in obtaining samples of abscess contents and blood and who supplied information throughout the investigation. It is regretted that lack of space prevents the mention of each gentleman by name.

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TYPES OF TUBERCLE BACILLI IN EQUINE TUBERCULOSIS.

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The horse in common with all our domesticated mammals is liable to contract tuberculosis naturally. Compared with the other ordinary livestock of a farm, horses suffer from tuberculosis much less frequently than cattle and pigs and more frequently than sheep and goats. Although not common in this country equine tuberculosis is by no means rare. In support of this statement the following authenticated instances in recent years may be quoted. In 1924, M'Fadyean described eleven cases in each of which the diagnosis was confirmed by cultivating tubercle bacilli from the lesions. In 1928 the writer published the results of typing the tubercle bacilli from eight cases collected during the period from August, 1922, to September, 1927. In 1929 Stableforth recorded the results in 13 cases which were examined in the years 1926-28. Previous to 1924 only five cases of equine tuberculosis had been examined bacteriologically—by F. Griffith for the Royal Commission on Tuberculosis (1911). The tubercle bacilli obtained in culture from these 37 cases were all of the bovine type culturally but only about half of those (34 in number) tested on animals exhibited the full virulence of standard bovine viruses, the rest falling in varying degree below the standard of virulence of the bovine type. The lesions found in these 37 cases were characteristic of equine tuberculosis, taking the form mainly of dense tumour-like masses in the organs and glands. In 1918 M'Fadyean described a case, so far unique, in which the gross lesions, which were enormously rich in acid-fast bacilli, were confined to the caecum and first part of the colon and resembled those produced in cattle by Johne's bacillus. A culture, however, obtained from the intestine proved to conform to the avian type of tubercle bacillus.

During the period from September, 1927, to November, 1935, I received material from 17 further cases of typical equine tuberculosis, from which I obtained cultures of tubercle bacilli and determined their type. Thus, the total number of cases of equine tuberculosis which I have investigated in Cambridge since 1922 is 25.

In this paper the results of my investigations of these 25 cases are summarised. The method of investigation throughout the series was as described in my previous paper (*loc. cit.*). Table I gives for each case the source of the material, an abstract of the *post-mortem* findings and the results of the bacteriological investigation.

TABLE I.

No. of Case.	*Sender and Date.*	*Description of Material.*	*Type of Bacillus.*	*Virulence.*
1.	Mr. S. Bennett, Cambridge. 17.8.22.	Long mediastinal gland enlarged, fibroid, contained pea-sized dense caseous nodules (t.b.+).	Bovine (Class I)	Below standard.
2.	Dr. A. Vanderheyden, Antwerp. 8.1.25.	Lung of old British army horse, resembled a "sarcome" with granulations, gritty foci present (t.b.++). Small nodules on pleura.	Bovine (Class II)	Below standard.
3.	Sir J. M'Fadyean, London. 16.10.25.	Mesenteric glands, dense, fibro-caseous (t.b.+++).	Bovine (Class I)	Standard.
4.	Mr. J. Pollard, Halifax. 19.9.25. Thro. I.A.P.	Spleen contained numerous firm homogeneous tumours up to 5 inches in diameter (t.b.+++).	Bovine (Class I)	Standard.
5.	Prof. S. H. Gaiger, Liverpool. 12.10.26.	Mediastinal gland much enlarged, fibro-caseous (t.b. +++). Lungs semi-solid with coalescing miliary tubercles. Spleen, a few tubercles. Mesenteric glands free.	Bovine	Below standard.
6.	Mr. F. C. Scott, Pontefract. 17.6.27. Thro. I.A.P.	Neck gland, fibro-caseous (t.b. ++++). Lung contained large fibro-caseous nodules and more numerous greyish-yellow nodules 2 to 3 mm. in diameter. Mesenteric glands also affected.	Bovine.	Standard.
7.	Slaughter-house, Cambridge. 2.6.27. Thro. I.A.P.	Mesenteric glands enlarged, dense fibro-caseous (t.b.+). Lungs thickly beset miliary tubercles. Spleen, large nodules. Perlsucht growths on pleura and pericardium.	Bovine	Below standard.
8.	Mr. J. R. Hewer, Swindon. 7.9.27. Thro. I.A.P.	Spleen, tuberc. perisplenitis and large fibro-caseous tumours (t.b.+++). Lungs and liver thickly beset miliary tubercles. Stiff neck during life.	Bovine (Class I)	Below standard.
9.	Mr. F. Frewin, Watford. 3.7.28. Culture thro. Mr. G. W. Dunkin.	Spleen, numerous tumours. Lungs enlarged and consolidated. Bronchial and mesenteric glands greatly enlarged. Extensive osteitis of 2nd, 3rd and 4th vertebrae (3-years-old cart mare).	Bovine (Class II)	Below standard.

TABLE I—*continued.*

No. of Case.	*Sender and Date.*	*Description of Material.*	*Type of Bacillus.*	*Virulence.*
10.	? ? ? ? Bedford. 24.9.28. Thro. I.A.P.	Spleen, numerous homogeneous tumours with no caseous areas (t.b.+).	Bovine (Class I)	Below standard.
11.	Mr. F. Beckett Dorset. 1.2.29. Thro. I.A.P.	Spleen, numerous tumours as in Case 10 (t.b. not found. Liver contained miliary tubercles.	Bovine	Standard.
12.	Mr. W. J. Leyshon, London. 18.4.29.	Mesenteric and bronchial glands enlarged and extensivelycaseous(t.b.++++). Lungs infiltrated grey tubercles and areas of necrosis. Tubercles on diaphragm and liver.	Bovine (Class I)	Below standard.
13.	Mr. H. Lomas, London. 15.3.30. Thro. Mr. W. J. Leyshon.	Lungs closely beset miliary tubercles (t.b. ++++); large calcified lesion base of left (not sent for examination). Bronchial glands enlarged, calcareo-caseous. Mediastinal glands, one hazel nut sized nodule. Spleen, "a tumour the size of an orange" contained pus. Mesenteric glands almost all enlarged, no statement as to condition. Bay gelding, aged 10 years.	Bovine (Class I)	Standard.
14.	Prof. S. H. Gaiger, Liverpool. 21.3.30. Thro. Mr. A. W. Stableforth.	Mesenteric glands much enlarged and extensively caseated (t.b.++++). Lungs largely replaced by sarcoma-like lesions. Very large gland under jaw.	Bovine (Class I)	Standard.
15.	Mr. W. S. King, London. 20.3.30. Thro. Mr. W. J. Leyshon.	Spleen and liver contained fibrous nodules up to 4 cm. in diameter (no t.b.). Mediastinal glands, one nodule. Lungs beset grey translucent tubercles. Draught mare, 5 years old.	Human	Below standard.
16.	? ? ? ? Denbigh. 17.2.31. Thro. I.A.P.	Spleen, many large nodules, some homogeneous, some caseo-purulent centrally (t.b.++++). Bronchial glands enlarged and caseating.	Bovine (Class I)	Standard.

TABLE I—*continued.*

No. of Case.	*Sender and Date.*	*Description of Material.*	*Type of Bacillus.*	*Virulence.*
17.	Mr. W. J. Leyshon, London. 27.2.31.	Mesenteric glands extensively caseous(t.b.++++). Mediastinal gland beset grey tubercles, aggregated into areas leaving little glandular tissue (t.b.+).	Bovine (Class I)	Standard.
18.	Mr. P. R. Turner, Bury St. Edmunds. 13.3.31. Thro. I.A.P.	Mesenteric glands enlarged, composed throughout of tough yellowish-white caseated substance (t.b. +++). Lungs practically solid, consisting mainly of dense homogeneous translucent grey tissue with discrete tubercles marginally.	Bovine (Class I)	Standard.
19.	Mr. J. G. Runciman, Cambridge. 15.9.31. Thro. I.A.P.	General tuberculosis primary in mesenteric glands which were densely fibroid and contained calcareous foci. Lungs beset as closely as possible with greyish-white miliary tubercles (t.b.+). Mediastinal glands, a few opaque points, no caseous areas. Spleen beset tubercles and small nodules, aggregated into large masses, one centrally purulent. Liver and omentum numerous tubercles.	Bovine (Class I).	Standard.
20.	Mr. F. C. Scott, Pontefract. 1.12.31. Thro. I.A.P.	Mesenteric glands enlarged, dense yellowish-white and homogeneous. Spleen contained a dense homogeneous nodule (t.b. not found micro.). A horse 12 years old.	Bovine	Below standard.
22.	Prof. S. H. Gaiger, Liverpool. 23.5.32.	Mesenteric glands greatly enlarged, together forming mass size of football, caseous and softened centrally (t.b. ++++). Spleen numerous nodules, up to golf-ball in size. Lungs dense miliary tuberculosis. No lesions liver or kidney. Pedigree shire mare, 14 years old.	Bovine (Class I)	Below standard.
23.	Dr. T. Hare, London. 6.6.32.	Spleen, large firm nodules (t.b.+). Lungs beset as closely as possible with miliary tubercles.	Bovine (Class I)	Standard.

TABLE I—*continued.*

No. of Case.	*Sender and Date.*	*Description of Material.*	*Type of Bacillus.*	*Virulence.*
24.	Mr. J. G. Runciman, Cambridge. 21.9.32. Thro. I.A.P.	Mesenteric (t.b.+++) and mediastinal glands extensively caseo-calcareous. Lungs enlarged and crammed miliary tubercles. Spleen enlarged and contained nodules. Mare 15 years old.	Bovine (Class I)	Standard.
25.	? ? ? ? Sussex. 11.1.34. Thro. I.A.P.	Spleen of mare 12 years old extensively replaced by semifibroid homogeneous tumours (t.b.+).	Bovine (Class I)	Below standard.
26.	Mr. J. G. Runciman, Cambridge. 15.11.34. Thro. I.A.P.	Mesenteric and mediastinal glands extensively caseo-necrotic, some calcification. Lungs enlarged and filled with nodules, discrete and in masses up to 1 inch in diameter. Gland pus contained incredible numbers of t.b. Mare 15 years old.	Bovine (Class II)	Below standard.

I.A.P. = Institute of Animal Pathology, Cambridge.

I am indebted to the various members of the veterinary profession mentioned in the table for the original material and for the *post-mortem* notes, and to colleagues (especially Mr. T. J. Bosworth) on the staff of the Institute of Animal Pathology, Cambridge, for handing over to me material sent to the Institute for confirmation of the diagnosis of tuberculosis.

REGIONAL DISTRIBUTION.

The cases it will be seen were derived from widely separated localities in England—London contributing six, Cambridge five, Liverpool three, Yorkshire three and Dorset, Wiltshire, Sussex, Bedfordshire, Suffolk, Denbighshire, Herefordshire, each one. The remaining one was an old British Army horse which was slaughtered in Antwerp.

PATHOLOGICAL DETAILS.

In most of the cases portions of the affected organs and glands were supplied in sufficient amount for description in the laboratory at Cambridge. In each of five cases (1, 3, 4, 10 and 25) the only material submitted for investigation was either a portion of the spleen or a single gland and nothing was said regarding the occurrence or not of lesions elsewhere in the body of the horse. In one instance (2) the tuberculous lesions were confined to the thoracic cavity, the lungs resembling a sarcoma with granulations

and the pleura showing many very small nodules. In two cases (11 and 20) abdominal structures only were mentioned as being affected. In the remaining 17 cases the tuberculosis was generalised, thoracic as well as abnormal organs being the seats of lesions. The lesions in the different horses exhibited the characteristic features of typical equine tuberculosis, which have been fully described by Nocard, M'Fadyean and others. As M'Fadyean has stated, the outstanding feature of tuberculosis in the horse is the frequency with which lesions other than those of the final miliary tuberculosis assume a rather tumour-like appearance. The most frequent seats of these tuberculomata are the mesenteric glands and the spleen, where they sometimes attain huge dimensions. In one of the cases (No. 22) in this series the enlarged mesenteric glands were fused into a mass which was as large as a football, and there are records in the literature of abdominal glandular masses of even larger size. The spleen is often very greatly enlarged and full of tumours which may reach a diameter of six inches. In this series the spleen was reported to contain nodules in 16 cases. The nodules varied in number and ranged in greatest diameter from less than 1 cm. up to more than 13 cm. They were rounded or knobby tumours, of a firm semi-fibroid consistence and white or yellowish-white colour; on section they were composed wholly or almost wholly of dense homogeneous fibroid substance usually free from any sign of caseation or calcification. In some instances the central parts had undergone softening and conversion into thick pus.

The lungs of two of the horses were well filled with nodules resembling those occurring in the spleen and in 14 other instances were enlarged and infiltrated by myriads of little tubercles which, where confluent, formed sarcoma-like masses. The liver, in which organ nodules are rarely so frequent or so prominent as in the spleen, was stated to contain miliary tubercles in three instances and fibrous nodules in one (Case 15, human infection). Tuberculosis of the pleura is much less frequent and conspicuous in the horse than in the ox and in this series was noted in only three cases (Nos. 2, 7 and 12). In two cases there was extensive tuberculosis of the neck vertebrae. The small intestine may be the seat of extensive tuberculous ulcers. In a case reported by Stableforth (*loc. cit.*) the only lesion found was a single ulcer, of peculiar appearance, in the small intestine, from which a fully virulent bovine strain was obtained.

With regard to the routes by which the tubercle bacilli entered the bodies of the horses the anatomical evidence was clearly in favour of the alimentary canal in eight of the cases. In these the glands in the mesentery showed more advanced tuberculosis than those in the thorax and were extensively caseated except in one instance where they were fibroid and contained calcareous foci. In four cases both the mesenteric and the mediastinal glands

were extensively caseous and the anatomical evidence regarding channel of infection was inconclusive, though it was probably the digestive tract since the lungs in these cases contained recent lesions only.

In one case (2) the lesions were confined to the thoracic cavity and in another (5) the mediastinal glands were fibro-caseous and the lungs were semi-solid with closely set miliary tubercles while the spleen contained only a few tubercles and the mesenteric glands were said to be unaffected. In these cases infection may have been respiratory but the evidence is not conclusive on that point. M'Fadyean is of the opinion from the results of numerous autopsies that the bacilli in equine tuberculosis usually enter the body by the digestive tract. He states that cases in which the situation of the lesions would suggest a primary lung infection are extraordinarily rare, if they occur at all, and ordinarily the lung tissues remain completely exempt from lesions for a long time before the animal has begun to lose condition.

Bacteriological Results.

Cultures of tubercle bacilli were obtained from the equine tuberculous tissues directly in 20 cases and through the guinea-pig in eleven cases. All the strains from 24 of the cases were highly dysgonic and exhibited the cultural characters of tubercle bacilli of the bovine type, the great majority resembling those bovine strains which grow with most difficulty on glycerine media, the rest growing only slightly better. The virulence of the bacilli from each of these cases was tested on the guinea-pig and the rabbit and, in four instances, on the goat also. The strains, two in number, from the remaining case (15) grew luxuriantly on artificial media and were identical culturally with the eugonic human type of bacillus.

Dysgonic Strains.—The bacilli from 12 of the 24 cases which yielded dysgonic strains proved fully virulent for the guinea-pig and the rabbit and, in one instance, for the goat as well. The virulence of the bacilli for the guinea-pig was judged by the results of subcutaneous inoculation of original material. Twenty-seven guinea-pigs were used and all died in from 39 to 95 days of typical caseo-necrotic general tuberculosis. In view of these results it was considered unnecessary to make further tests on the guinea-pig with cultures of the bacilli. The virulence of the twelve viruses for the rabbit was tested by the subcutaneous inoculation of cultures, the doses ranging from 1 to 10 mg. Fifteen rabbits were used and all died of general tuberculosis typical of that produced by standard bovine bacilli and of full severity except in one in which the disease was slightly less extensive than usual. The duration of life of twelve of the rabbits ranged from 45 to 96 days and in the remaining three

was rather prolonged, namely, 122, 129 and 189 days respectively. The bacilli were recovered in culture from the rabbit which died 189 days after inoculation and from the rabbit in which the disease was not of full severity and which died in 96 days. The two strains were tested by subcutaneous inoculation each on one rabbit and produced severe general tuberculosis typical of that which follows similar inoculation of fully virulent bovine bacilli. Two strains (3 and 4) were each tested on a goat by subcutaneous inoculation of 10 mg. of culture. One goat died in 20 days of miliary tuberculosis of the lungs; the other died in 26 days of general miliary tuberculosis.

The tubercle bacilli from the remaining twelve cases fell below the standard of virulence of bovine bacilli both for the rabbit and the guinea-pig. Preliminary indication that the bacilli in these twelve cases were not of normal virulence was afforded by the results of the inoculation of original material into guinea-pigs. Twenty-four guinea-pigs were inoculated with tuberculous tissues from the twelve cases and none developed the typical caseo-necrotic rapidly fatal form of tuberculosis seen in all the guinea-pigs inoculated with material from the twelve virulent cases. Four of the 24 guinea-pigs were injected intraperitoneally and developed a less acute form of tuberculosis than guinea-pigs similarly inoculated with virulent bacilli. Twenty guinea-pigs were inoculated subcutaneously and five of these died from causes other than tuberculosis or were killed for the purpose of obtaining cultures of the bacilli. The tuberculosis in these animals was slight in extent although their duration of life ranged from five to eleven weeks. The remaining 15 guinea-pigs died or were killed after periods ranging from 87 to 997 days. The results are summarised in Table II.

Four of these strains were also tested as to their virulence for the guinea-pig by the inoculation of cultures of the bacilli. The results given in Table III are in agreement with those produced by the inoculation of the original material in these cases and show clearly that the bacilli were less virulent for this species of animal than standard bovine bacilli.

The virulence of the twelve atypical strains was tested on rabbits by the subcutaneous, and in four instances also by the intravenous inoculation of cultures. The results summarised in Table IV confirmed those obtained in guinea-pigs with these strains, none of the rabbits developing the severe rapidly fatal form of progressive tuberculosis which almost invariably follows similar inoculation of fully virulent bovine bacilli. The effect produced in the rabbits varied widely and ranged from moderate general tuberculosis to disease limited to the seat of inoculation.

The culture of one virus (20) produced results which indicated that its virulence was only slightly below the standard of bovine viruses. At the other end of the scale of virulence is Case 10,

TABLE II.

No. of Case.	*No. of Guinea-pig.*	*Equine Material Inoculated.*	*Duration of Life in Days.*	*Result.*
2.	4270	Mediastinal gland	D.284	Severe fibroid G.T.
8.	5035	Spleen	D.156	Chronic G.T.
	5036		D.193	Chronic severe G.T., lesions fibro-caseous.
	5037		K.230	Slight G.T., lesions fibroid.
10.	5192	Spleen	D.200	Slight local T. only.
	5193		K.578	Very slight G.T.
	5194		K.997	Moderate G.T., lesions fibroid.
12.	5314	Bronchial gland	D.134	Slight G.T., lesions fibroid.
	5313		D.413	Chronic G.T., lesions fibro-caseous.
	5315		D.434	Very slight G.T.
22.	6082	Mesenteric gland	D.87	Glands except nearest only slightly enlarged and not caseous. Liver and spleen beset grey tubercles.
	6083		D.183	Extensive necrosis liver and spleen. Portal and bronchial glands dense and fibroid.
25.	6694	Spleen	D.160	Severe G.T., lesions fibroid.
26.	6861	Mesenteric gland	D.111	Chronic G.T., little caseo-necrosis.
7.	—	Mixed lesions	K.89	Grey miliary tubercles in spleen, liver and lungs; fibrosis and caseous softening of some glands.
	5018	Pus from sternal gland of above guinea-pig	K.138	Slight enlargement and fibrosis of some glands and enlarged Malphigian bodies of spleen.

TABLE III.

No. of Case.	*No. of Guinea-pig.**	*Dose of Culture.*	*Duration of Life in Days.*	*Results.*
1.	3523	0·1 mg.	D.40	Atypical subacute G.T.
	3524	0·1 mg.	D.117	Chronic G.T.
	3525	0·1 mg.	D.151	Chronic G.T., severe.
5.	4837	between 0·1 and 1·0 mg.	D.133	Numerous grey foci, liver and spleen.
	4838		K.194	Chronic fibroid G.T., retrogressive.
9.	5182	„	D.226	Local T. only; death from pneumonia.
	5197	1·0 mg.	D.134	Chronic G.T.
	5198	1·0 mg.	D.165	„
	5199	1·0 mg.	D.185	„
10.	5508	1·0 mg.	D.82	Mild G.T., liver and spleen rather closely beset grey foci.
	5509	1·0 mg.	D.110	Local T. only; death from pneumonia.

*All subcutaneous except G.P.3523 which was intraperitoneal.

which produced a local lesion only in a rabbit inoculated subcutaneously and very slight retrogressive tuberculosis of the lungs and kidneys in a rabbit inoculated intravenously (dose 0·01 mg.). The results in guinea-pigs with the original material and culture of this case also clearly showed that the virulence of the bacilli was much below the standard (*see* Tables II and III) of bovine strains. Case 12 also produced results which indicated that the virulence of the bacilli was very low. The remainder of the strains gave rise in at least one rabbit to tuberculosis more widespread than that which follows similar inoculation of human bacilli but definitely less severe than after virulent bovine bacilli. It is impossible to arrange these strains in order of virulence since the results obtained with individual strains were very irregular, in some instances varying nearly as much as for the whole group.

The virulence of two of the strains, namely 1 and 8, was tested on the goat, the former on two kids five months old, the latter on one kid six to eight months old, the dose in each instance being 10·0 mg. subcutaneously. Strain 1 produced severe acute results in each kid. Strain 8 gave rise to a chronic form of general tuberculosis with death in 102 days.*

No attempt was made by prolonged passage through the bodies of animals to raise the virulence of any of the anomalous bovine strains but some were recovered in culture from rabbits which had been used to test the original virulence of the bacilli. Four strains thus recovered were tested on rabbits by subcutaneous inoculation. One strain was again recovered from the second test (or first passage) rabbit, the total duration of residence in rabbit tissues in this instance being 173 days. None of the rabbits inoculated with recovered cultures developed more severe disease than any inoculated with the original cultures. In a further instance (5) the bacilli were passed directly in tissue emulsions from an original guinea-pig, which died in 45 days of mild general tuberculosis, into a rabbit which when killed 164 days later showed moderate general tuberculosis, nodules being found on the pleura. An emulsion of these pleural nodules produced in a rabbit chronic general tuberculosis severe in kidneys only, the animal dying in 124 days. In each of two further instances (1 and 7) material from a guinea-pig which had been inoculated with original material was passed on into another guinea-pig. In each instance the tuberculosis in the second guinea-pig was not more severe than that in the first. The results of these experiments, while demonstrating the stability of the modified equine strains after short periods of residence in rabbits and guinea-pigs, at the same time confirm the conclusion that the virulence of these bacilli was much below the standard of bovine viruses. F. Griffith (*loc. cit.*) carried out passage experiments, some in

*For complete *post-mortem* notes see Reference 1.

calves, others in rabbits, with two of the five strains of equine origin which he investigated for the Royal Commission on Tuberculosis; these strains were culturally bovine but were less virulent than standard bovine bacilli for all species of animals. In one experiment virulence was increased along some but not all of its several branches. In the second experiment the virulence was not increased along two lines but was increased along a third.

Apart, however, from the decisive proof of identity with the bovine bacillus, such as would be afforded by the increase to standard virulence as a result of animal passage, the original properties of these twelve viruses sufficiently indicate their relationship with the bovine group of tubercle bacilli. They exhibited the cultural characters of bovine bacilli and, with the exception of Case 10, they produced in rabbits, and in calves and goats where these were used, tuberculous disease anatomically similar to that set up by bovine bacilli but not so rapidly fatal.

These modified equine strains are akin to the modified bovine strains which have been obtained so frequently from the human subject, especially in tuberculous conditions affecting the skin. In the case of some modified lupus strains it has been conclusively proved that strains originally fully virulent lost their virulence during residence in the skin tissues. There is little doubt that in equine tuberculosis also the infection, at any rate in the great majority of instances, is primarily with fully virulent bovine bacilli and that the modification of virulence occurs in the tissues of the horse.

Eugonic Strains.—From the spleen of Case 15 two strains of tubercle bacilli were obtained through the guinea-pig which were identical culturally with the eugonic human type of bacillus. The bacilli were of low virulence for the rabbit and distinctly less virulent for the guinea-pig than standard human tubercle bacilli. The results in this case were fully reported in 1936.

Number and Characters of the Bacilli in the Lesions.

M'Fadyean (*loc. cit.*) drew attention to the remarkable fact that the extremes of rarity and abundance of the tubercle bacilli in the lesions are represented in different cases of tuberculosis of the horse, and, one may add, sometimes in different parts of the same tuberculous lesion. In my series tubercle bacilli were abundant in 14, moderately numerous in one and scanty or not found in nine cases. As a general rule the firm homogeneous tumours without areas of softening are very poor in bacilli, prolonged microscopical search of film preparations often failing to reveal a single organism and cultures producing only few colonies. On the other hand, caseous substance in glands or the collections of pus occasionally found within the splenic tumours frequently contain the bacilli in great abundance. It is of

historical interest to mention that Koch remarked upon the enormous quantities of tubercle bacilli in one of the four cases of equine tuberculosis which he investigated. As already mentioned, the numbers of bacilli may vary widely in different parts of the same lesion. For example, a small collection of caseo-pus in the midst of a large splenic tumour was extremely rich in bacilli, whereas the homogeneous semi-fibroid parts contained them very sparingly.

As M'Fadyean has also observed the tubercle bacilli in equine tuberculous lesions, particularly in those cases where they are extremely abundant, are generally distinctly longer than those present in bovine lesions.

With regard to the degree of virulence of the tubercle bacilli in relation to the numbers of organisms in the tissues of the horse one finds that the proportion of virulent to attenuated strains is as three to two among the cases in which the tubercle bacilli were abundant whereas among the cases with scanty tubercle bacilli the proportion of virulent to attenuated was as one to two.

Summary.

The total number of cases of equine tuberculosis investigated bacteriologically in England is now 55, this number including the five cases examined by F. Griffith (1911) for the Royal Commission on Tuberculosis.

The tubercle bacilli cultivated from these 55 cases belonged to the bovine type in all but two instances, one of them yielding a strain of human, the other a strain of avian type.

The bovine strains all exhibited the cultural characters of the more dysgonic members of the bovine type and rather more than half of them were fully virulent for the test animals. The rest were less virulent than standard bovine bacilli for all the species of animals tested, the degree of attenuation varying from slight to considerable.

Table IV.
Intravenous and Subcutaneous Inoculation Experiments on Rabbits with the Attenuated Dysgonic Strains.

No. of Virus.	*No. of Rabbit.*	*Material Inoculated.*	*Dose in mg.*	*Duration of Life.*	*Result.*
1.	2798*	Culture from from gland of horse	0·01	D.104	Chronic moderate gen. mil. tuberc. (G.M.T.)
	2799*		0·01	D.96	Severe G.M.T.
	2800		25·0	K.222	Slight G.M.T.
	2801		24·0	D.99	Slight G.T.
	3034		10·0	D.92	G.T. very widespread but slight in individual organs and glands.
	2873	Culture lung R.2799	10·0	K.111	Slight T. lungs, kidneys and and eye.
	2874		15·0	K.112	Lungs, a dozen submil. tubercles. One kidney, one tubercle.

TABLE IV—*continued.*

No. of Virus.	*No. of Rabbit.*	*Material Inoculated.*	*Dose in mg.*	*Duration of Life.*	*Result.*
2.	3110	Culture from lung	15·0	D.92	Slight G.M.T. One eye and a lachrymal gland tuberculous.
	3111		15·0	D.88	Slight G.M.T.
	3118		3·0	D.74	Moderate G.M.T.
	3200	Culture kidney R.3118	20·0	D.99	Moderate G.T.; one eye tuberculous.
	3245	Culture kidney R.3200	20·0	D.108	Moderate G.T.
5.	3273	Pus from gland G.P. inoc. O.M.	—	K.164	Moderate G.T.
	3304	Pleural nodule R.3273	—	D.124	Chronic G.T., severe in kidneys.
	3268*	Culture from gland of G.P.	1·0	D.19	Acute G.T.
	3280		3 primary cultures	D.103	Moderate G.T.
	3286		15·0	K.114	Slight T. of lungs.
7.	3320	Culture from organs	20·0	D.96	Slight T. of lungs.
	3321		20·0	D.106	Moderate G.T.; one knee-joint tuberculous.
8.	3347	Culture from spleen	20·0	K.111	Slight miliary T. of lungs, spleen, kidneys and some glands. T. of eyes, malar bone and knee-joints.
9.	3402	Culture from spleen	12·0	D.130	Slight G.T.
	3403		10·0	D.164	Chronic moderate G.T.
10.	3438*	Culture from spleen	0·01	K.193	Slight retrogressive T. lungs and kidneys only.
	3439		16·0	K.193	Small local cystic lesion only.
12.	3466	Culture from bronch. gl.	20·0	K.201	Slight G.M.T.
20.	3643*	Culture from spleen	0·01	D.27	G.M.T.
	3644		10·0	K.158	Chronic moderate G.T.; projecting nodules in kidneys and early perlsucht.
22.	3715	Culture from mesent. gl.	20·0	K.181	Slight T. of lungs and kidneys, many bones and joints affected.
	3716		20·0	K.183	Lungs and kidneys, each 1 tubercle. T. of 3 bones, head of each radius and a tarsal bone.
25.	3953	Culture from equine spleen	14·0	K.141	Chronic G.T. widespread.
	4033	Culture pop. gl. Rabbit 3953	10 to 15	K.117	Chronic G.T.
26.	4052	Med. gl culture	1 P.C.	K.153	Chronic G.T. not severe.
	4054	Mes. gl. ,,	1 P.C.	K.151	Slight G.T.

G.M.T. = General miliary tuberculosis.
* Intravenous Inoculations.

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STAPHYLOCOCCUS ANTITOXIN IN THE BLOOD AND MILK OF COWS AND OTHER ANIMALS.

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Observations on bovine mastitis caused by toxigenic staphylococci (*Staph. aureus*) have recently been recorded (Minett, 1937). At the same time, studies were made of the antitoxin content of the blood and milk of cows and other animals under various conditions, and the results of this work are given below.

Technique.

Toxins were prepared according to the method outlined in a previous paper (Minett, 1936). Plates of 0·8 per cent. beef heart agar, pH 7·4, made up with 1·0 per cent. Witte peptone and 0·25 per cent. NaCl were sown and incubated for 36 hours at 37° C. in an atmosphere containing 25 per cent. CO_2. 1·0 c.c. saline was then added to each plate of 10·0 c.c. agar, the medium chopped, and as much fluid as possible pressed out and passed through a Berkefeld filter. Such filtrates are hæmolytic for rabbit and for sheep cells if α toxin is present and for sheep cells in the cold after previous incubation at 37° C. if β toxin is present.

In titrating blood serum for antitoxin, the toxic filtrates were diluted as little as possible (never more than 1 in 20) and 0·5 c.c. volumes were mixed with serum in amounts falling by 50 per cent. differences. The tubes, after standing on the bench for 30 to 60 minutes, were incubated in a water-bath at 37° C. for 30 minutes; 0·1 c.c. of a 10 per cent. suspension of washed red cells was added, the tubes were then returned to the bath for an hour, being shaken at the end of the first half-hour. Readings were taken after one hour's standing on the bench and again after standing overnight in the cold, the smallest amount of serum required for neutralisation being noted.

Milk whey (obtained by the use of rennet) was tested for α antitoxin by mixing 0·2 c.c. volumes of whey with 0·5 c.c. volumes of toxin in increasing dilution; after a preliminary incubation, rabbit cells were added as before, and the neutralisation point noted.

In titrating for α antitoxin, rabbit red cells were used; in the few cases where an attempt was made to determine the β unitage, an α-free toxin* and sheep cells were used. In all cases the unitages of the serum or whey were determined by comparison with standard sera.* The antitoxin values obtained by these methods were of course approximate only, but as a general rule the labour of attempting greater precision was not justified.

Antitoxin in the Blood of Normal Cattle, Sheep and Goats.

The α and β unitage of cow serum has been referred to previously (Minett, 1936). The α unitage is generally below 7·0 and the β unitage usually does not exceed $0{\cdot}45\gamma$.† Further

* Obtained from the Wellcome Physiological Research Laboratories.

† As no agreed international standard has yet been formulated for β antitoxin, values are still being expressed as γ units per c.c.

particulars have now been obtained. In the first place, the α or β unitage of samples of blood serum from 107 male cattle, from 110 sheep and 29 goats was determined, the results being shown in Table I.

TABLE I.

ANTITOXIN IN BLOOD SERUM OF CATTLE, SHEEP AND GOATS.

Species.	*Samples Tested.*	*α antitoxin (units per c.c.).* <1·1	1·1	2·2	4·5	9·0	18·0
Cattle (male)	104	77	13	13	1	–	–
Sheep (male)	57	51	2	3	1	–	–
Sheep (female)	53	30	12	10	1	–	–
Goats (less than 3 years)	12	8*	3	1	–	–	–
Goats (3 years to over 6 years)	17	1	1	7	5†	2	1

Species.	*Samples Tested.*	*β antitoxin (units per c.c.).* <0·2γ	0·2γ	0·4γ	0·9γ	1·8γ
Cattle (male)	107	102	5	–	–	–
Sheep (male)	56	53	2	1	–	–
Sheep (female)	50	43	7	–	–	–
Goats (less than 3 years)	11	5*	5	1	–	–
Goats (3 years to over 6 years)	15	2	8	3†	1	1

TABLE II.

ANTITOXIN TITRE OF BLOOD SERUM OF MALE CATTLE IN RELATION TO AGE.

Age (in Years).	*Number.*	*Number Showing at Least 1·5 Units.*	*α antitoxin (units per c.c.).* 1·5	2·2	4·5	9·0	18·0
<3	33	7	–	5	2	–	–
3– 4	12	4	–	1	1	1	1
4– 6	20	13	1	3	4	4	1
6– 8	14	6	–	4	1	–	1
8–10	3	3	1	1	–	1	–
>10	4	3	–	–	2	1	–

The cattle and sheep sera were taken at the abattoir, the cattle and female sheep being estimated at three or four years old and the male sheep at 12 to 18 months old. It is seen that the samples from female sheep usually reacted to a higher titre than those from male sheep, though owing to the difference in age true comparison is not possible. Serum from goats over three years of age reacted more strongly than that from younger goats. All were females except one—a young male—in cells marked *, and two—aged males—in cells marked †.

In Table II are shown α antitoxin titrations of sera from a further series of 86 male cattle of which the exact age or almost exact age was known. There is evidence of direct relationship

between age and antitoxin titre. Thus, of 33 animals under three years of age, only seven had $> 1{\cdot}5$ units and of these only two had as much as 4·5 units. On the other hand, of 53 animals three or more years old, 29, *i.e.*, more than half, had at least 1·5 units and of these 18 had at least 4·5 units.

That the antitoxin titre of the serum of female cattle similarly increases with age may be seen from Table III. The 159 animals belonged to one herd and all the bloods were taken within a period of 48 hours.

TABLE III.

ANTITOXIN TITRE OF BLOOD SERUM OF CATTLE IN RELATION TO NUMBER OF CALVINGS.

Number of Calvings.	*Number of Animals.*	*Number Showing at Least 3·7 Units.*	*α antitoxin (units per c.c.).* 3·7	7·5	15·0
0*	33	1	0	1	0
1	34	15	10	4	1
2	40	21	14	6	1
3	38	30	19	10	1
4 or 5	14	8	4	3	1
	159	75	47	24	4

* Pregnant animals, mostly 2 to 2½ years old.

RELATIONSHIP BETWEEN BLOOD ANTITOXIN AND UDDER INFECTION WITH *Staph. aureus*.

Information on this question was collected mainly in two ways; firstly, by injecting *Staph. aureus* into the healthy udder and seeing whether a rise in blood antitoxin would follow, secondly, by observing the blood antitoxin levels of a number of cows, a proportion of which had naturally infected udders.

Cow *Annabel*, about three months before the end of her first lactation, was injected *via* the teat canal of the left hind quarter with a 24-hour broth culture of an udder staphylococcus. Two injections of 1·0 c.c. were given at an interval of nine days. The changes which took place in the quarter were described previously (Minett, 1936). The disturbances of the quarter and its secretion, due to the injections, were transient and comparatively slight, but they reappeared immediately after the second calving, which took place seven months later, during four of which the cow was dry. The blood antitoxin curve is shown in Chart 1. The injections caused a slight but definite immediate rise in blood antitoxin, but after calving the rise became much more pronounced.

Data concerning the influence of natural infection of the udder with *Staph. aureus* on the blood antitoxin titre are shown in Table IV. Fortunately, the number of cows found to be infected was nearly the same as the number in which this organism was

CHART 1. COW ANNABEL.

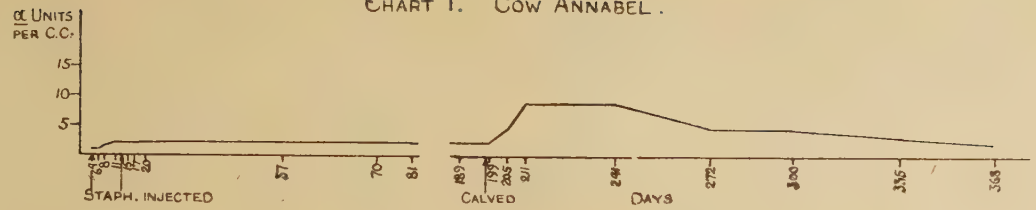
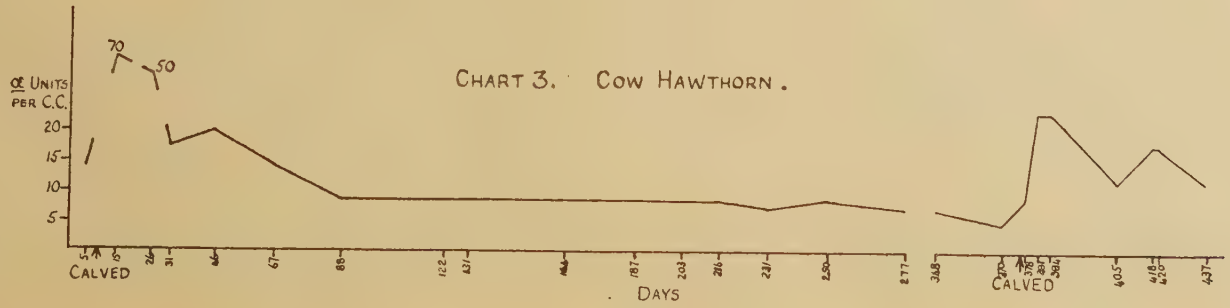


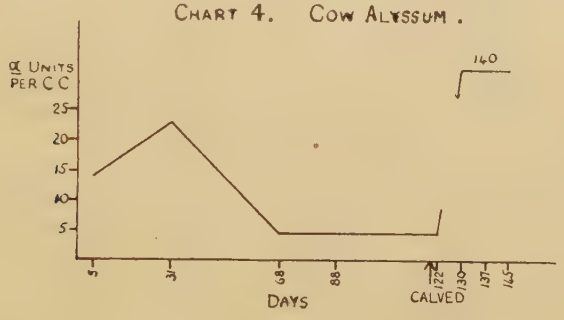
CHART 2. COW HOPE.

CHART 3. COW HAWTHORN.



not detected. Actually, the information to be derived from such data is not entirely clear-cut, partly because of the real difficulty of being sure that an udder is not infected with toxigenic staphylococci, and partly because, if the infection is light or not sustained, any considerable antitoxin rise would not be expected. The first point to be noted in Table IV is that in three-fourths of the animals with inappreciable amounts of circulating antitoxin *Staph. aureus* was not found in the udder, whereas about two-thirds of the animals with infected udders had at least 2·3 units antitoxin per c.c. Another point to be noted in Table IV is that as age increases there is a gradually increasing proportion of cows with infected udders.

CHART 4. COW ALYSSUM.



The result with cow *Annabel* suggested that the pronounced rise in circulating antitoxin which occurred after calving was associated with renewed growth of staphylococci in the refunctioning udder. In connection with this point, samples of blood were taken at intervals from three cows whose udders were known to have been infected with *Staph. aureus* for several months. The results are shown in Charts 2 to 4, comparison being made in this case with standard serum, A14956 (75 α units per c.c.). Charts 3 and 4 illustrate the enormous rise in α antitoxin which may follow calving. Apart from such rises, the titre tended to remain fairly steady at 4·6 to 9·3 α units per c.c. With cow *Hope* (Chart 2), samples were unfortunately not taken soon enough after calving to show whether a rise had actually occurred.

Further observations on the same point were as follows. A number of cows were bled shortly before calving and again several times at intervals up to two or three months after calving, and their serum titrated for α antitoxin. The animals in Group I were first-calvers and *Staph. aureus* could not be detected in the milk; those in Group II were second-calvers, except the first three which were first-calvers, and *Staph. aureus* was isolated from the milk. The titration results, expressed as units of

TABLE IV.

RELATIONSHIP OF UDDER INFECTION TO PRESENCE OF α ANTITOXIN IN THE BLOOD.

Units of α Anatoxin per c.c. Serum.	*Calvings.*									
	First.		*Second.*		*Third.*		*Fourth or More.*		*All Calvings.*	
	Staph. +	*Staph.* —	*Staph.* +	*Staph.* —	*Staph.* +	*Staph.* —	*Staph.* +	*Staph.* —	*Staph.* +	*Staph.* —
<1·2	9	31	9	29	7	9	3	5	28	74
1·2	3	7	14	22	11	16	5	6	33	51
2·3–4·6	0	5	15	13	40	9	15	3	70	30
9·2	2	5	6	8	13	13	9	5	30	31
15 or over	2	1	7	2	7	1	9	1	25	5
Total cows	16	49	51	74	78	48	41	20	186	191
	65		125		126		61		377	

antitoxin per c.c., were as follows, the titre shown after calving being the highest observed.

Group I.								
Before calving ...	<0·5	<0·5	<0·5	<0·5	0·5	0·75	1·0	1·3
After calving ...	1·5	<0·5	<0·5	<0·5	0·5	1·0	1·0	2·0
Group II.								
Before calving ...	<0·5	<0·5	0·5	2·0	2·0	3·0	3·2	9·0
After calving ...	0·75	1·5	2·0	6·4	6·4	8·0	4·8	9·0

Thus, in Group I an appreciable rise can be said to have occurred in only one of the eight animals, whereas in Group II an appreciable rise occurred in at least five of them. With the last animal in Group II there was no rise from the initial titre of 9·0 units, but in this case repeated examination of the milk showed that a heavy infection of the R.H. quarter prior to calving had been overcome during the dry period.

Effect of Injections of Staphylococcus Toxoid in Cows and Sheep on the Antitoxic Power of their Blood Serum.

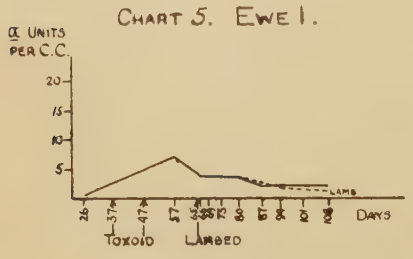
In the case of man there is a good deal of knowledge as to the stimulating effect of toxoid on antitoxin production. Thus, Dolman (1935) obtained 5x to 10x and occasionally higher increases after eight injections of toxoid; Murray (1935) obtained 8x increases after a series of four injections. Domesticated animals would, of course, be expected to react in much the same way. With cows and particularly with sheep, the use of toxoid has practical possibilities in lessening the systemic effects of udder infections, provided a good and reasonably lasting antitoxin response can be induced.

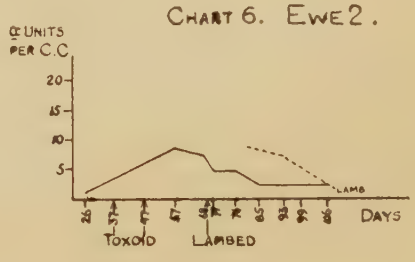
The toxoid used in most of the experiments described in this and the succeeding section was obtained from a strain of good toxigenicity, isolated from the bovine udder. No special tests of the toxoid for antigenicity or combining powers were, however, carried out. Agar filtrates (Berkefeld), prepared in the usual way and lytic for rabbit cells at a dilution of at least 1 in 1,000, were treated with formalin at a final concentration of 0·2 per cent. and incubated at 37° C. until lysis was absent or slight at a dilution of 1 in 10; two days usually sufficed for this purpose.

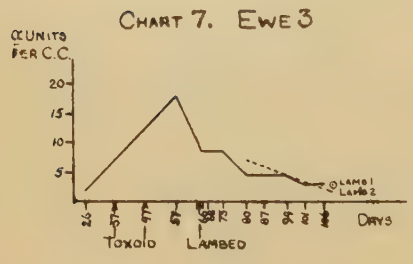
Experiments with Sheep.

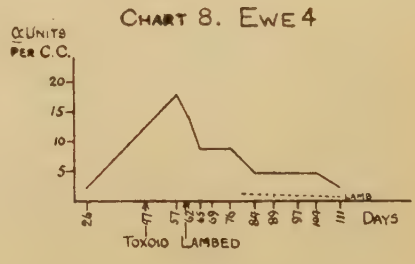
Six ewes (1 to 6) in the later stages of pregnancy were injected subcutaneously with toxoid and three similar ewes (seven to nine) were left untreated. Three of the former were given a single dose of 10·0, 7·5 or 5·0 c.c. respectively, while in three others a second similar dose was given ten days later. Each ewe produced one or two lambs at time and the antitoxin titres of the blood serum of both ewes and lambs are shown in Charts 5 to 13. It will be seen that after a preliminary rise the amount of antitoxin gradually fell, though it was still higher than before six to eight weeks after lambing. The increase was

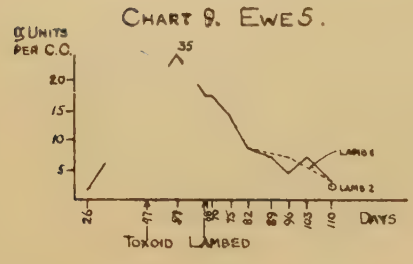
usually about four to eight times, but in this series no comparison can be made between the effects of one and of two doses. The titre, however, did rise to a higher level in those sheep (3, 4 and 5) whose sera contained an easily measurable amount of natural antitoxin before lambing, *e.g.*, in sheep 5 the serum titre was 32 times higher than the initial titre and reached a value half that of the serum being used as standard, *viz.*, A14956. This is, no doubt, a secondary stimulus effect by the

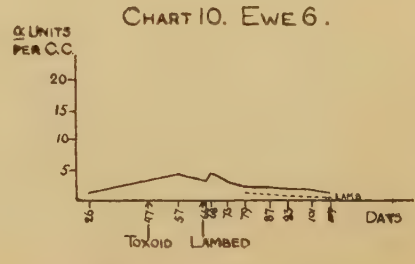












toxoid such was noted by Bryce and Burnet (1932) in rats, and by Parish, O'Meara and Clark (1934) in human beings showing deep-seated staphylococcus infections. Incidentally, it may be noted that *Staph. aureus* was isolated from the milk of ewes 4 and 5, one month after lambing. The control ewes failed to show any corresponding increase of antitoxin over the period of lambing, in spite of the fact that the serum of ewe 8 contained measurable amounts of natural antitoxin before lambing. It is also seen from the charts that the serum of lambs from ewes 1 to 6 and from control ewe 7 contained

antitoxin, the titres being highest in lambs born of ewes 2 and 5. With the lambs from ewes 4 and 6 antitoxin was measurable for 20 days and 15 days by the technique used, but in most of the lambs antitoxin was detectable for at least 40 days after birth.

Experiments with Cows.

Cow *Mayfly* in her first lactation was given subcutaneously two injections of 10·0 c.c. toxoid at an eight-day interval;

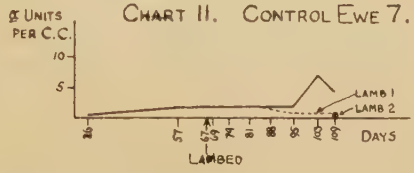


CHART 11. CONTROL EWE 7.

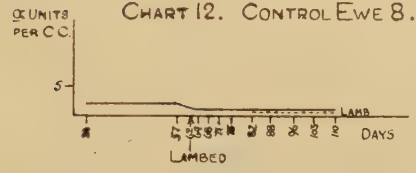


CHART 12. CONTROL EWE 8.

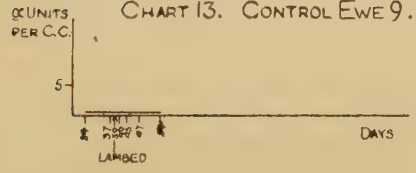


CHART 13. CONTROL EWE 9.

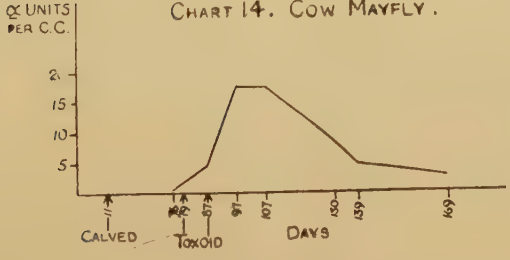


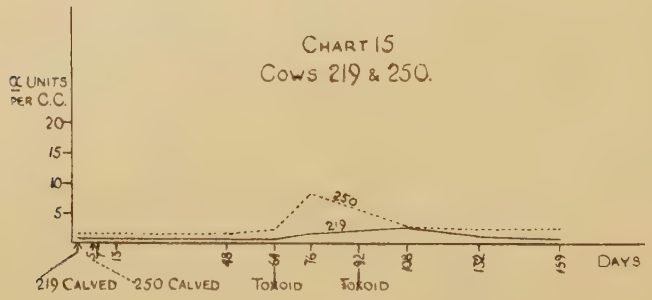
CHART 14. COW MAYFLY.

titrations of her blood serum are shown in Chart 14. As will be seen, there was a sharp rise in antitoxin, the titre being increased at least 16x and still being slightly raised after two-and-a-half months.

Cows 219 and 250 were treated with toxoid prepared from three strains, two of human and one of bovine origin, recognised to be good producers of toxin. Two injections were given at a 28-day interval, the amounts being 5·0 and 8·0 c.c., and 10·0 and 10·0 c.c. respectively, and the toxoid being diluted with

four or five volumes of saline just before injection. Titrations of the blood serum are shown in Chart 15, from which it will be seen that in neither case was a rise of more than 4x registered.

Finally, eleven cows in milk, all of which were infected in the udder with *Staph. aureus*, were given hypodermic injections of toxoid. Ten of them were given three injections of 2·0, 5·0 and 5·0 c.c. undiluted toxoid at ten-day intervals. The initial α unitage of their serum ranged from less than 1·1 to 2·3 units, except in one case, where it was 4·6 units. The increase in titre due to the injections was 4x in one, at least 4x in one, 5x in two, at least 5x in one, 8x in four, at least 8x in one. Two further injections of toxoid, each of 10·0 c.c., failed to raise the



titre of the first animal more than four times the original titre. The remaining cow was treated with five injections of 2·0, 2·0, 5·0, 8·0 and 10·0 c.c. undiluted toxoid prepared from the homologous strain. In this case the serum titre was raised 2x only, but the initial titre was comparatively high, namely 14·5 units. Incidentally, in none of these animals were staphylococci eliminated from the udder as a result of toxoid treatment; such a result would not in fact be anticipated.

Staphylococcus Toxin and Antitoxin in Milk Whey.

It was of interest to examine milk whey for toxin and antitoxin, and, in cases where antitoxin was present, to determine its quantitative relationship to blood antitoxin, as well as the conditions in which it appeared in the whey.

Toxin.

Whey prepared from freshly taken milk of quarters affected with chronic staphylococcus mastitis has not been found to be lytic for rabbit or sheep cells. On the other hand, toxin can

be demonstrated in the udder secretion from cases of gangrenous mastitis. Three samples of secretion were tested. The first was lytic for rabbit cells at a dilution of 1 in 20, produced an area of necrosis 2·5 cm. in diameter on a rabbit's skin at a dilution of 1 in 16 and slight necrosis on guinea-pig skin at a dilution of 1 in 8. In two other instances the toxin content was determined more exactly by direct titration with rabbit and sheep cells, by intradermal injection in rabbits and by finding the LH dose (volume of secretion neutralised by one unit of standard antitoxin). The results were as follows :—

Case.	*Reciprocals of Final Active Dilution with*			*L.H. Dose*
	Rabbit Cells.	*Sheep Cells.*	*Rabbit Skin.*	*(α Toxin).*
2	150	<25	32	1·0 c.c.
3	200	300	40	0·7 c.c.

The staphylococcus isolated from case 2 was subsequently found to produce α toxin only, while that from case 3 produced both α and β toxin. For comparison, it may be said that the LH dose of a good culture toxin should be 0·1 c.c. or even less.

Antitoxin.

The presence of antitoxin in whey can often be demonstrated. In the first place, samples of wheys from the individual quarters of 20 cows were tested in an attempt to correlate the antitoxin titre of the whey with udder infection with *Staph. aureus*. There was no definite evidence of such a correlation, but there *were* indications that the concentration of antitoxin in the whey was related to disturbance in the quarter from any cause, *e.g.*, from streptococci, since wheys from milks showing an excessive deposit on centrifuging or an excessive alkalinity contained a larger amount of antitoxin. This is in conformity with the work of Jones (1926), who found that when the udder tissue is inflamed excessive amounts of blood serum filter into the milk. It is also known that in cows infected with *Br. abortus* the agglutinin titre of the whey is likely to be high in quarters showing disturbance. Table V gives illustrative results of a few cases where there was very definite disturbance in at least one quarter.

TABLE V.

Cow.	Quarter.	Condition of Milk.	Serum Content of Whey.	Units of α Antitoxin in 1 c.c. of Blood Serum.	Units of α Antitoxin in 1 c.c. of Whey.	Br. abortus Agglutinin titre of Blood Serum.	Br. abortus Agglutinin titre of Whey.
1	L.F.	N	2·0×	4·7	0·05	1,280	200
	L.H.	A, 0·5, inn. strep.	3·0×		0·30		800
	R.F.	N	2·5×		0·05		400
	R.H.	N	1·5×		0·05		100
2	L.F.	SA, 0·01	2·5×	9·3	0·25	320	50
	L.H.	A, 0·02, β-hæm. strep.	6·0×		1·80		180
	R.F.	SA, 0·01	3·0×		0·25		50
	R.H.	N	5·0×		0·50		120
3	L.F.	N	6·0×	9·3	0·50	320	80
	L.H.	SA, 0·01 blood	6·0×		0·90		160
	R.F.	N	3·0×		0·10		40
	R.H.	N	5·0×		0·25		80
4	L.F.	N	0·4×	4·7	0·10	1,280	15
	L.H.	N	0·9×		0·10		20
	R.F.	N	0·8×		0·05		20
	R.H.	N, inn. col.	3·0×		0·15		240
5	L.F.	N	2·0×	4·7	0·10	5,120	40
	L.H.	N, inn. col.	2·5×		0·10		20
	R.F.	N, β-hæm. strep.	3·0×		0·15		160
	R.H.	N	2·5×		—		40
6	L.F.	N, β-hæm. strep.	2·5×	2·3	0·20	80	5
	L.H.	N, *Staph. aureus*	1·0×		—		0
	R.F.	SA, 0·025, inn. β-hæm. strep.	6·0×		0·40		60
	R.H.	N	2·0×		0·40		5

N = normal reaction and no centrifuge deposit; S = slight; A = alkaline; inn. col. = innumerable colonies on plate; the figures refer to volumes of deposit estimated in c.c. on centrifuging 10·0 c.c. milk.

I am indebted to Mr. E. J. Pullinger for determining the agglutinin titres and the serum content of the whey. The agglutinin titres are expressed as reciprocals of the final dilutions. The figures for the serum content of the whey are hypothetical and represent the relative proportions of serum in the quarters of individual cows, as calculated by a precipitin method using the optimal proportions technique. × represents the influence of some factor which inhibits precipitation and which, though varying from cow to cow, is approximately the same in quarters of the same animal.

Influence of Toxoid Injections on the Antitoxin Titre of the Whey.

Antitoxin, produced in response to injections of toxoid, can sometimes be detected in the whey. Tables VI and VII illustrate results obtained with the ewes and with cow *Mayfly* referred to above.

TABLE VI.

ANTITOXIN IN WHEY AFTER TOXOID INJECTIONS.

Ewe and (udder)	*Half.*	*Units of a Antitoxin in 1·0 c.c. of Whey on Day (after Lambing).*										
		1	3	6	9	12	17	22	27	31	36	40
1	L.	—	4·2	1·0	—	0·5	0·2	—	0·3	0·2	—	0·2
	R.	—	11·2*	8·4*	—	—†	0·2	—	0·2	0·2	—	0·3
2	L.	—	1·0	0·5	0·5	0·3	0·2	—	0·2	—	0·2	—
	R.	—	1·0	0·5	0·5	0·3	0·2	—	0·2	—	0·2	—
3	L.	—	8·4	0·7	0·5	—	0·4	0·3	—	0·3	0·3	—
	R.	—	4·2	1·0	0·5	—	0·3	0·3	—	0·3	0·3	—
4	L.	—	—	0·5	0·5	0·25	—	0·2	—†	—	0·2	0·1
	R.	—	—	—†	—†	1·4	—	0·3	0·2	—	0·2	0·2
5	L.	—	1·4	0·7	0·5	—	0·6	0·3	—	0·3	0·4	—
	R.	—	1·4	1·0	0·5	—	0·4	0·3	—	0·3	0·4	—
6	L.	—	1·0	0·2	0·35	—	0·3	0·3	—	0·3	0·2	—
	R.	—	2·1	—†	0·35	—	0·3	0·2	—	0·2	0·2	—
7	L.	8·4	0·2	—	0·25	—	0·3	0·2	—	0·2	0·3	—
	R.	5·6	0·2	—	0·25	—	0·3	—†	—	0·2	0·3	—
8	L.	—	0·2	0·2	0·1	0·2	—	0·1	0·1	—	0·1	0·1
	R.	—	0·25	0·2	0·2	0·1	—	0·1	0·1	—	0·1	0·1
9	L.	—	0·1	—	0·05	—	0·05	—	—	—	—	—
	R.	—	0·2	—	0·05	—	0·05	—	—	—	—	—

Two injections of toxoid were given prior to lambing. * milk contained pus. † milk clotted on standing and no whey could be obtained.

TABLE VII.

ANTITOXIN IN WHEY AFTER TOXOID INJECTIONS.

Cow and Quarter.	*α Antitoxin (Units per c.c.) on Day (after Calving).* 79	87	97	107	127	139	169
Mayfly							
L.F.	0·1	0·35	1·4	0·7	0·2	0·2	0·2
L.H.	0·2	0·35	1·4	0·7	0·2	0·2	0·2
R.F.	0·1	0·35	1·4	0·7	0·2	0·2	0·2
R.H.	0·1	0·35	1·4	0·7	0·2	0·2	0·2

Days (after calving) correspond to those shown in Chart 14.
Toxoid was injected on days 79 and 87.

In the case of the ewes, the titre of the whey was raised for a few days after lambing and then declined; the decline, however, was more gradual in the toxoid-treated ewes (1 to 6) than in the control ewes (8 and 9). The sheep for this experiment had been unselected, and it so happened that the serum of ewe 7 was found after lambing to contain some antitoxin due to natural infection; hence, this ewe behaved much like ewes 1 to 6 in respect of the antitoxin content of its whey. As stated above, antitoxin could also be demonstrated in the blood of the lambs from ewes 1 to 7.

With cow *Mayfly*, the injections caused a considerable rise in the antitoxin titre of the whey, the effect lasting for at least three months. In this case the udder was normal and antitoxin was excreted uniformly by the four quarters. That this may not always happen is evident from the results with three other cows with normal quarters, where after injections of toxoid the blood serum titre was raised 2x, 4x and 4x the original, but without any detectable rise in the whey titre.

RELATIONSHIP BETWEEN THE ANTITOXIN TITRES OF BLOOD SERUM AND OF COLOSTRUM OR MILK WHEY.

Observations on this point are set out in Table VIII. The approximate unitage of the serum is given, while the amounts of antitoxin in the colostrum or whey may be roughly determined from the ratios shown at the head of the table. In normal quarters the amount of α antitoxin in the whey is most commonly from 1/80 to 1/40 that in the blood serum taken at the same time; with colostrum, on the other hand, the amount is much higher and often exceeds that present in the blood serum. The figures also suggest that in cows with higher blood serum titres the whey/serum ratio tends to fall below the mode, which is situated within the range 1 : 40 to 1 : 60, and *vice versa*.

TABLE VIII.

RELATIONSHIP BETWEEN α ANTITOXIN OF BLOOD SERUM AND OF COLOSTRUM OR WHEY FROM NORMAL QUARTERS.

Serum Antitoxin (*Units per c.c.*).	*Colostrum / Serum antibody ratio.* 7·7 : 1 *to* 5·7 : 1	3·2 : 1 *to* 2·2 : 1	2 : 1 *to* 1 : 1	1 : 1·5 *to* 1 : 3	1 : 4 *to* 1 : 8	1 : 10 *to* 1 : 20	1 : 30
23–18	–	1*	3	–	4	–	–
11–7·5	–	5	10	5	2	3	3
5·6–2·8	–	1	11	5	7	5	–
2·3 or less	5	9	28	17	8	3	3
	5	16	52	27	21	11	6

Total = 138.

Serum Antitoxin (*Units per c.c.*).	*Whey / Serum antibody ratio.* 1 : 1 *to* 1 : 2	1 : 4 *to* 1 : 8	1 : 10 *to* 1 : 20	1 : 30	1 : 40 *to* 1 : 60	1 : 80	1 : 120 *to* 1 : 160	1 : 350
23–18	–	–	–	—	–	2	9	6
11–7·5	–	–	4	2	41	22	24	–
5·6–2·8	–	3	3	17	115	33	10	–
2·3 or less	1	3	5	27	4	–	–	–
	1	6	12	46	160	57	43	6

Total = 331.

* This sample of colostrum—derived from a cow with chronic staphylococcus mastitis—contained 58 units antitoxin per c.c.

DISCUSSION

It has been shown that the staphylococcus antitoxin titre of the blood increases with age in man, being low in infants a few weeks old and rising considerably during the first six years of life (Bryce and Burnet, 1932). The increase of titre with age also occurs in horses, sheep and swine (Ramon, Richou and Descazeaux, 1935), while in this paper the same has been shown to be true of goats and cattle. In cows, evidence points to the lactating udder as being one of the sites—often perhaps the main site—of staphylococcus growth, and hence a fruitful stimulus for antitoxin production. This evidence is as follows. In the first place, a survey of a group of cows (Table IV) pointed to a direct relationship between circulating antitoxin and infection of the udder with *Staph. aureus*. Again, the proportion of animals carrying antitoxin in the blood in demonstrable amounts is very much higher in first calvers than in animals pregnant for the first time, although the difference in age between the two groups may, on an average, be less than a year (Table III). After calving, a considerable increase in blood antitoxin can sometimes be demonstrated, presumably as a result of multiplication of staphylococci in the freshly functioning udder. Presumably also,

considerable increases would be most likely to occur in animals whose udders were extensively infected, though the outcome would also most probably be influenced by the individual's capacity to respond to the antigen and by the titre prior to calving. Finally, the injection of toxigenic staphylococci into the healthy lactating udder is followed by a rise in circulating antitoxin, a rise which tends to be most marked after the next parturition, when the staphylococcus count in the milk is high. In order to throw light on the problem from another angle, blood samples from a number of adult bulls were examined for antitoxin (Table II). The fact that of 53 adult bulls ten had 9 to 18 units α antitoxin per c.c. serum makes it clear that, as might have been expected, in cattle the udder is not the only site of staphylococcus growth.

Staphylococcus antitoxin can also be detected at times in milk whey. The uneven distribution of antitoxin in different quarters and its relationship to disturbance in the quarter brings to mind the question of the source of antibodies in milk, *e.g.*, *Br. abortus* agglutinins. Several observers, *e.g.*, Smith, Orcutt and Little (1923), Bang and Bendixen (1932), Niermann (1932), have noted that such agglutinins reach a higher titre in whey from quarters showing disturbance than in whey from normal quarters. The experiments of Smith, Orcutt and Little (1923) led them to believe that *Br. abortus* agglutinins might be produced locally in the udder following invasion by the organism, and other workers, *e.g.*, Balozet, Cordier and Ménager (1934), have come to a similar conclusion. An alternative explanation, however, is more probably the correct one, namely, that agglutinins present in the whey are always derived from the blood and that their higher titre in quarters with disturbance is due to increased vascular permeability in such quarters and is unrelated to infection of the udder tissue with *Br. abortus*. The reasons in favour of this explanation are, shortly, as follows. It has been shown by Crowther and Raistrick (1916) that lactoglobulin is chemically identical with cow serum globulin, while Wells and Osborne (1921) by means of anaphylaxis experiments also found these two proteins to be identical. Jones (1926) and Jones and Little (1933) found that during normal lactation a slight but well-defined quantity of blood protein passes into the udder; that, as mentioned above, in the milk of quarters with mastitis the amount of protein in increased, the increase being due to serum globulin; and that, in consequence, mastitis milk reacts to a higher titre with a cow serum precipitin than normal milk. Previous to this, it had been shown by Orcutt and Howe (1922) that *Br. abortus* agglutinins in colostrum or in the blood of newly-born calves which have suckled are associated with the globulin fraction. The most reasonable view, therefore, is that lacto-globulin and agglutinins that may be associated with it are derived from the blood of the mother. In the same connection, it is now well

established that in ruminants and swine antibodies easily pass from the blood into the colostrum. This was proved by Brieger and Ehrlich (1893) for tetanus antitoxin produced by injecting pregnant goats with toxin, by Famulener (1912) for the haemolysin produced in goats by injecting sheep erythrocytes, by Little and Orcutt (1922) for *Br. abortus* agglutinins in cows, by Mason, Dalling and Gordon (1930) for lamb dysentery antitoxin in ewes and for diphtheria antitoxin in a cow following active immunisation with the appropriate toxin, and by Nelson (1932, 1934) in sows which had recovered from experimental vaccinia. Again, in bovine tuberculosis, the occurrence of complement-fixing antibodies in the blood serum and milk whey has been noted by a number of observers. Karsten (1933, 1935) has clearly shown that a positive reaction in the case of milk whey simply means that the animal is suffering from advanced tuberculosis of an internal organ such as the lungs, and by no means implies that tuberculous lesions are present in the udder or that tubercle bacilli are present in the milk. In such instances the milk was frequently abnormal in some way. It is also possible, though much less frequent, for tubercle bacilli to be present in the milk of cows whose milk whey gives a negative reaction in the complement test.

The observations recorded in the present paper indicate that staphylococcus antitoxin is no exception to the rule. When the antitoxin content of the blood is increased beyond a certain point, *e.g.*, by treatment with staphylococcus toxoid, it is sometimes possible to demonstrate antitoxin in the milk of normal quarters. If there is a reaction in a quarter to organisms such as streptococci, the milk contains serum in excess, and antitoxin—as well as *Br. abortus* agglutinins, if the animal is infected with that organism—can then be more readily demonstrated. The use of a precipitin test with milk whey was in fact suggested by Jones (1926) as a means of revealing abnormality in the gland, and it seems that titration of whey for staphylococcus antitoxin might be used for the same purpose, provided there was a certain level of antitoxin in the blood.

Summary.

(1) It has been found that in cattle (male and female) and in goats, as in some other animals, there is a direct relationship between the age of the animal and the staphylococcus antitoxin titre of its blood.

Evidence is brought forward to show that the presence of antitoxin in the blood of cows is determined in part by infection of the udder with *Staph. aureus*, and in the same connection it may be noted that the incidence of udder infection with *Staph. aureus* also increases with age.

(2) As would be expected, in cows and sheep injections of

staphylococcus toxoid lead to an increased concentration of antitoxin in the blood, and this is reflected at times by an increase of antitoxin in the milk.

(3) Staphylococcus toxin can be demonstrated in the udder secretion of acute cases of staphylococcus mastitis.

(4) In the milk whey from normal quarters the amount of antitoxin present is usually 1/80 to 1/40 of that in the blood serum, whereas in colostrum the quantity of antitoxin may equal or even exceed that present in the blood. When the quarter is inflamed, *e.g.*, as a result of streptococcus infection, the amount of staphylococcus antitoxin in the whey rises.

(5) The facts brought forward in this paper have a bearing on the general question of the source of " natural " antitoxin in blood and of antibodies in milk.

I am indebted to the Director of the Wellcome Physiological Research Laboratories for supplies of standard antitoxin.

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CHANGES IN THE SEDIMENTATION RATES OF THE RED BLOOD CORPUSCLES DURING TRYPANOSOME INFECTIONS.

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It has been known for a very long time that an increased rate of sedimentation of the red blood corpuscles accompanies various pathological conditions. In fact, so great a volume of literature has accumulated on this subject that no attempt can be made to review it here. Of later years efforts have been made to determine the factors governing this phenomenon of decreased stability and certain workers have suggested formulæ correlating the sedimentation rate with the blood composition and physical constants. From the work on the parallelism between plasma protein changes and sedimentation velocity it is now agreed that an increase of either the fibrinogen, the total globulin or euglobulin contents, or a decrease of the albumin/globulin ratio will tend to reduce the suspension stability of the red blood cells. In spite of these and other findings, Fahraeus (1929) in an extensive review of the subject is forced to the conclusions " that the mechanisms having to do with the stability of the blood must be regarded as very complicated and far from clear " and " the problem seems to hinge upon the manner in which the globulin increase changes the interface between the red corpuscles and the plasma or, let us say, the surface of the red cells. Is it by increasing the surface tension, or by reducing the negative electric charge of the corpuscles or finally by depriving their surface colloids of absorbed water?"

Increased sedimentation velocities have been reported for a large number of very different types of disease, so this phenomenon by itself can be of little diagnostic significance, although its presence can often be taken as valuable corroborating evidence. It is known that in trypanosome infections increases of the plasma total globulin and euglobulin may be expected, together with increases in the surface tension and viscosity of the blood and a reduction of the charge on the red cells. If it were not previously known, it could therefore be readily deduced, that in trypanosome infections there is an increased sedimentation rate of the red cells.

In the course of some detailed nutritional and biochemical studies on trypanosomiases in the larger farm animals the opportunity was taken of studying the sedimentation rates of the red cells. It was hoped that some correlation might be found between the sedimentation velocity and the blood chemical changes but these hopes were not realised.

The normal sedimentation rates in healthy cattle and sheep differ considerably from those in healthy donkeys, and it was decided to measure the increased rates in these three classes of

stock, using two species of trypanosomes which are responsible for serious economic losses, namely *T. congolense* and *T. brucei*.

Experimental Procedure.

The sedimentation rate was measured by a modification of Westergren's method, and the results are expressed as mm. of clear plasma resting over the sedimented red cells after one hour, when 1 ml. of oxalated blood was standing in a capillary tube of such dimensions that the blood occupied a 257 mm. length of the tube.

In previous publications (French and Hornby, 1934, 1935, 1, 2) dealing with the influence of nutrition in trypanosomiases full details of the animals and their management and infections have been given, and so it is only necessary to give here a brief review of the animals reported upon below.

Oxen 2134 and 3143 were placed upon a sub-maintenance hay ration, and ox 3143 was inoculated with a virulent strain of *T. congolense*. An acute infection resulted and the animal reached a very distressing state by the time of crisis in the 20th week after inoculation.

Oxen 2863 and 2785 were given a maintenance hay ration and ox 2785 infected with the same strain of *T. congolense*. In this case the infection was of a chronic type and the animal was never so seriously ill as ox 3143. Crisis was reached in the 27th week after inoculation and was succeeded by a period of 19 weeks when the animal was in liveweight equilibrium and approaching a state of premunition.

Sheep W and M were inoculated with the above strain of *T. congolense*, sheep W being on a sub-maintenance hay diet whilst sheep M received a maintenance ration of hay and cake. Both sheep suffered from an acute infection, sheep W dying in the 12th week and sheep M in the 18th week after inoculation.

Sheep Umoyo, Dunia and Imani, were given a maintenance hay ration and sheep Umoyo and Imani were inoculated with *T. brucei*. Acute infections resulted and these animals died in the sixth and fifth weeks respectively after inoculation.

Sheep Tumbo and Majani were given a productive ration of hay and concentrates and sheep Tumbo was inoculated with the above virulent strain of *T. brucei*. He died in the twelfth week after inoculation.

Donkeys 3, 17 and 22, were fed an *ad lib.* ration of hay. Donkey 3 was inoculated with the above *T. brucei* strain and died five weeks later after an acute infection. Donkey 17 was inoculated with a strain of *T. congolense* and suffered from slight fever but no loss of weight. However, in the eighth week after inoculation *T. brucei* suddenly appeared in the blood and immediately the animal began to exhibit marked clinical symptoms of trypanosomiasis. Death took place in the eleventh week after the initial inoculation.

The results are presented in Tables I and II ;—

TABLE I.

RED CELL SEDIMENTATION RATES IN CATTLE AND SHEEP BLOOD DURING *T. congolense* INFECTION.

Weeks after Inoculation.	*Ox* 2134 (*Control*).	*Ox* 3143 (*Infected*).	*Ox* 2863 (*Control*).	*Ox* 2785 (*Infected*).	Sheep W (*Infected*).	Sheep M (*Infected*).
0	1·0	1·4	1·0	0·8	1·5	1·8
2	—	1·1	—	1·0	2·0	1·5
4	1·2	1·2	1·1	1·1	3·0	3·0
6	—	1·3	—	1·2	5·4	4·2
8	1·1	1·8	1·2	1·4	6·2	4·5
10	—	2·1	—	1·6	10·4	6·1
11	—	—	—	—	10·5	8·3
13	1·2	2·6	1·1	2·2	Died	—
15	—	3·0	—	2·5	—	—
17	—	—	—	—	—	10·1
19	1·3	3·6	1·2	2·8	—	Died
20	—	4·0	—	—	—	—
24	—	3·1	1·3	2·6	—	—
31	1·2	1·8	1·1	2·1	—	—
35	1·1	1·2	1·2	1·5	—	—

TABLE II.

RED CELL SEDIMENTATION RATES IN SHEEP AND DONKEYS DURING *T. brucei* INFECTION.

Weeks after Inoculation.	*Sheep Tumbo* (*Infected*).	*Sheep Majani* (*Control*).	*Sheep Umoyo* (*Infected*).	*Sheep Dunia* (*Control*).	*Sheep Imani* (*Infected*).	*Weeks after Inoculation.*	*Donkey* 22 (*Control*).	*Donkey* 17 (*Infected*).	*Donkey* 3 (*Infected*).
0	1·7	2·1	1·5	1·2	1·8	0	158·2	158·5	124·1
3	4·6	1·5	9·5	1·2	4·2	2	157·6	172·6	163·9
6	12·0	1·7	15·5	1·8	15·0	4	158·9	180·4	202·0
9	25·0	2·1	Died	—	Died	6	—	194·3	222·3
—	Died	—	—	—	—	9	—	196·5	Died
—	—	—	—	—	—	—	—	Died	—

There is a marked difference in the sedimentation rates of normal healthy cattle and sheep blood when compared with donkeys' blood. Also in every case of infection the rate of sedimentation was increased, but although the increase was not very pronounced in cattle and sheep blood it was most marked in the rapidly sedimenting donkey blood. Sheep blood showed a greater increased settling rate after infection than did cattle blood. Once crisis is passed in cattle suffering from *T. congolense* infections the rate of sedimentation of the red cells returns slowly to its normal value.

As stated earlier, the diagnostic value of the sedimentation rate cannot be very great, but it is obvious that the marked increase in the red cell sedimentation rates in "fly-struck" equines could be used as an indicator even though trypanosomes were not apparent in blood smears.

Summary.

Figures are given showing the big difference in the sedimentation rates of normal healthy cattle and sheep blood when compared with donkeys' blood. Increased sedimentation rates follow infection with *T. congolense* and *T. brucei,* but the increase is small in cattle, slightly greater in sheep, and very much more marked in donkeys' blood.

I would like to express my thanks to Mr. H. E. Hornby (Director of Veterinary Services) for permission to carry out and publish this work.

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THE ELECTRICAL CONDUCTIVITY OF BLOOD AND SERUM IN ACUTE TRYPANOSOMA BRUCEI INFECTION.*

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Introduction.

In acute fatal trypanosomiasis the physico-chemical changes which are produced in the host are highly important, and it is possible that such changes are responsible for the death of the animal. Since it is well known that fatal trypanosomiasis is associated with hypoglycæmia, and since the electrical conductivity of the infected blood has not been determined up to the present time, this study was undertaken to determine whether any marked change exists in the conductivity of the serum or blood of nagana rats during the progress of the disease, in comparison with normal rats.

Extensive application of conductivity measurements have been made in the past by a number of investigators on various biological fluids and serums from normal and diseased sources of human and animal origin. Among some of the best known workers in this field, whose publications it would be well to consult in this connection, are S. C. Brooks, P. Lasseur, F. McClendon, W. J. Osterhout and G. N. Stewart.

The majority of investigators have used high frequency alternating current methods for the determination of conductivity; only a few have used direct current methods. While most of the other investigators preferred platinised electrodes, G. N. Stewart states that he obtained good results without platinising the electrodes of the conductivity vessel.

Methods and Procedure.

A. *Inoculation of the Animals.*—About 80 rats (*Rattus norvegicus*) were inoculated with *Trypanosoma brucei* at various intervals. Each series consisted of 10 to 25 animals, all of which were inoculated within one hour. The electrical conductivity determinations were made on serum at various stages of the disease. Animals were etherised and their heart blood was removed and defibrinated. This defibrinated blood was immediately centrifuged for one hour at about 2,400 r.p.m. A

* This work was completed during the early part of 1930.

The writer wishes to express his indebtedness to Professor A. Ferguson, of the Department of Electrochemistry, the University of Michigan, for various apparatus which he placed at the writer's command during the conductivity measurements. Appreciation is also due to Mr. Hill who prepared the conductivity cell.

few samples were supercentrifuged at about 7,200 r.p.m.* The serum was then carefully transferred by means of a Pasteur pipette into a sterile test tube and its electrical conductivity determined.

Normal rats were bled as controls in conjunction with the nagana rats, and their blood was subjected to identical procedures.

A limited number of normal rabbits were bled through the carotid artery. Their blood was defibrinated and conductivity measurements were obtained on both the defibrinated blood and the serum therefrom.

A normal chicken was bled from the carotid artery into ice-cooled sterile test tubes and the blood centrifuged at once. This plasma was then transferred into other sterile test tubes. The animal was bled primarily for tissue culture experimentation; however, one tube of plasma was permitted to clot and the serum was removed for conductivity measurements.

A sample of horse serum was obtained from Parke & Davis, Co., and its conductivity was likewise determined. The serum had been in cold storage for slightly more than two months before the determination was made upon it. Apparently no preservative had been added.

Tyrode solution, which is used in connection with the growing of vertebrate tissues, was also subjected to conductivity measurements.

B. *Determination of Conductivity.*—For the determination of conductivity, the Kohlrausch High Frequency Current Bridge method was employed.

In view of the fact that the amount of blood obtained from any given rat is very small (the maximum being in the neighbourhood of 8 c.c.), a small conductivity cell of one cubic centimetre capacity was constructed.

This conductivity cell (illustrated in Figure I) consists of a central part which receives the fluid of which the conductivity is to be determined, and two side arms which serve as mercury bridges in the circuit. The cell space proper holds about one cubic centimetre of fluid, and the two platinum electrodes projecting into it are spaced one centimetre apart. The platinum discs are supported on platinum wires which project through a seal of lead glass into the side arms. The side arms are filled with mercury, which forms the electrical connection between the platinum wires and the copper connecting wires leading through the stoppers in the openings of the side arms. The lower part of the apparatus is designed so as to be removable for cleaning purposes.

* The centrifuge used is made by Burmeister and Wain, of Copenhagen. The machine has a maximum speed of 8,000 revolutions per minute, each 100 revolutions being indicated by a bell when desired.

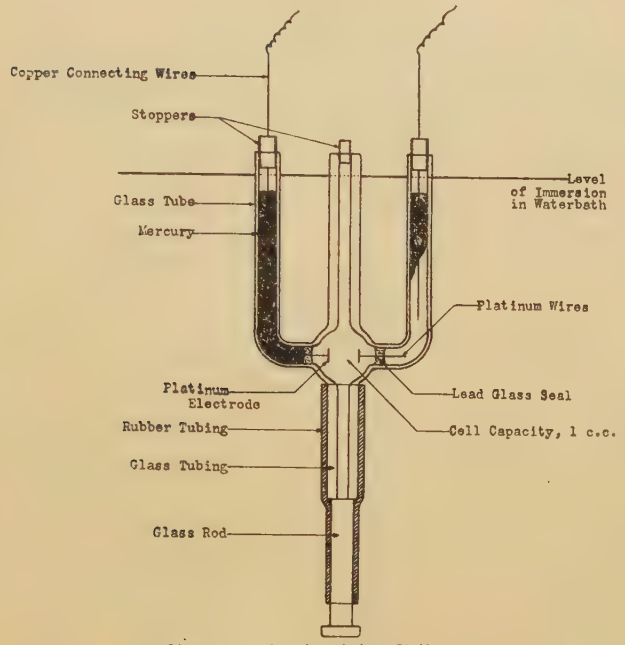

FIG. 1. Conductivity Cell.

The electrodes of the cell were platinised in order to eliminate polarisation effects. Before and after use, the cell was cleaned thoroughly with conductivity water.

The conductivity cell was immersed into a well regulated conductivity bath, the temperature of which was kept at 25·000° C., and the first measurement was taken ten minutes after immersion. The cell constant K was determined at frequent intervals by using N/50 KCl solution whose conductivity was 0·002768 mhos.

When a sufficient amount of material was available duplicate determinations on a given sample of serum or blood were made. In all cases care was taken to expel any air bubbles which were present in the fluid. To avoid the possibility of leaving any conductivity water in the cell it was washed with the solution whose conductivity was to be determined next.

In almost all cases readings were taken twice with different resistances Rb in the circuit, and the average was used as a final number.

DISCUSSION AND SUMMARY.

The results obtained on normal and nagana infected serums and defibrinated blood samples are summarised in Tables I and II. To conserve space, not all of the conductivity measurements were included in the tables, nor were the details of the measurements included. This was considered permissible because of the uniformity of the results.

Table I.
Conductivity of Serum and Blood in Acute *Tr. brucei* Infection.

Rat No.	*Days of Infection.*	*No. of Trypanosomes p.m.f. before Blood Centrifugation.*	*Determination Made on.*	*Conductivity in 10^{-4} mhos.*
302	1	None found	Serum	119·36
303	2	2	,,	112·50
310	3	10	,,	117·71
312	4	50	,,	122·13
314	4	50	,,	122·13
315	5	100	,,	122·13
319	5	100	,,	119·50
320	5	100	,,	119·16
321	5	100	,,	106·23
322	5	100	,,	122·14
323	5	100	,,	117·72
324	5	100	,,	113·97
325	5	100	,,	113·62
326	5	100	,,	122·14
327	5	100	,,	116·88
328	6	200	,,	122·40
329	6	200	,,	125·02
330	6	200	,,	125·43
331	6	200	,,	122·90
332	6	200	,,	107·94
333	6	200	,,	112·38
335	6	200	,,	119·41
336	6	200	Blood	70·58
337	6	200	,,	69·46
338	6	200	,,	73·98
339	6	200	,,	67·34

An examination of the tables reveals the following facts:—

1. A distinct difference in conductivity is noted in the case of nagana and normal defibrinated rat blood. The conductivity of nagana defibrinated blood varies from $67{\cdot}34 \times 10^{-4}$ to $73{\cdot}98 \times 10^{-4}$, while that of normal blood varies from $47{\cdot}96 \times 10^{-4}$ to $57{\cdot}76 \times 10^{-4}$. The conductivity of normal defibrinated rabbit blood is $67{\cdot}51 \times 10^{-4}$.

2. There is no appreciable difference in the conductivity of nagana rat serum (freed from *Tr. brucei*) as compared with normal rat serum, even in the terminal stages of the disease. From the time of bleeding to the end of the experiment, the samples were subjected to identical external conditions.

Slight variations in both nagana and normal animals are probably due to experimental deviations, and variations in the animals themselves. However, the results on the whole are quite uniform, but no specific interpretation can be attempted. The conductivity of these serums varies from $106{\cdot}23 \times 10^{-4}$ to

Table II.

Conductivity of Normal Serum and Defibrinated Blood.

Kind of Animal.	*Specimen Number.*	*Determination made on*	*Conductivity in 10^{-4} mhos.*
Rat	200	Serum	122·36
	203 and 204	„	123·68
	303 and 304	„	116·31
	305 and 307	„	117·95
	341	„	117·90
	342	„	113·62
	343	„	119·16
	344	„	116·63
	345	„	117·30
	346	„	112·52
Rabbit	1	„	152·90
	2	„	148·20
	3	„	120·30
	4	„	133·10
	205	„	119·36
	206	„	122·35
	207	„	124·80
	208	„	125·49
	209	„	127·11
	212	„	117·95
Chicken	—	„	148·34
Horse	—	„	116·63
Rat	340	Blood	47·96
	343	„	53·35
	346	„	54·62
	347	„	57·76
	348	„	55·60
Rabbit	213	„	67·51

$125{\cdot}43 \times 10^{-4}$ for nagana rat serum, and $112{\cdot}52 \times 10^{-4}$ to $123{\cdot}68 \times 10^{-4}$ for normal rat serum.

3. Normal serums of rabbit, chicken and horse give a conductivity of the same magnitude as normal and nagana rat serums. Tyrode solution used in connection with tissue culture work has a conductivity of $157{\cdot}59 \times 10^{-4}$. This solution, however, contained one more gramme of monovalent sodium salt per litre of solution than is used in the usual formula.

4. It is evident that the conductivity of normal and nagana serums is always higher than that of serum which contains blood cells or *Tr. brucei*. This lower conductivity of the cells is apparently due to their physico-chemical structure and the relative impermeability of the cell wall to the ions.

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ABSTRACTS.

FIFTH PROGRESS REPORT OF THE FOOT-AND-MOUTH DISEASE RESEARCH COMMITTEE, 1937.*

THE Report covers approximately the research work carried out during the five-year period ended September 30th, 1935.

When the Committee was appointed in 1924, it was known that foot-and-mouth disease was caused by a filtrable virus, but little was known of the length of time which the virus could survive under various conditions, or of the relative importance of methods of spread other than directly from one infected animal to another ; or of the possible occurrence of the disease in wild rodents or other animals ; or of the possibility of inducing an effective, active or passive immunity.

The programme of the Committee during the recent period was designed to obtain further information regarding the character and properties of the virus itself, and secondly to solve problems having a practical bearing on effective action to be taken against the disease.

The properties of the virus have been studied particularly in relation to its resistance to physical and chemical changes in the environment ; the different types, their stability and inter-relationship have been examined ; the susceptibility to the disease of all the ordinary domestic animals, including the dog and cat, and also of the rat, mouse, rabbit, hedgehog, vole, ferret and various species of birds investigated. Further knowledge has been gained of the extent to which the important domestic animals are infective at different stages of the disease, and of the conditions under which transmission takes place from one animal to another. Further study has been given to the presence of the virus in various organs and tissues and in such secretions as milk, saliva and excreta. Diseases which are related to or resemble foot-and-mouth disease, namely, vesicular stomatitis and vesicular exanthema of swine, have been extensively studied.

It has been suggested that the virus might be introduced into this country as a contamination of milk powder which had been made from the milk of infected cows, but the investigation undertaken by the Committee showed that in the process most commonly used the temperature reached would be sufficient to destroy the activity of the virus, and attempts to infect pigs by feeding them with imported milk powder all had negative results.

The possibility of the persistence of the virus in imported sweetbreads (calf thymus) was also considered and a series of experiments was made to find out the temperature reached in the interior of sweetbreads during cooking and also the final activity or otherwise of virus previously introduced into these organs. The results indicated that when the organ was placed in cold water which was then heated, there was a small margin of safety in the case of the smaller sweetbreads if the time taken to raise the water to boiling point was 12 to 13 minutes and boiling was continued for 5 minutes, but with larger specimens weighing 320 g., this procedure was not quite enough to ensure killing of the virus.

Attempts to propagate the virus of the disease in eggs failed completely whereas the virus of vesicular stomatitis could be readily cultivated in this way.

*To be purchased directly from H.M. Stationery Office. Price 7s. net.

During the period covered by the Report, much attention was given to the question of immunity and to the occurrence of so-called types of virus, knowledge of which is important in tracing the sources of infection and also in endeavours to produce immunity by the use of the specific vaccine or serum.

Vallée (1926) distinguished two types, O and A, and three years later Trautwein claimed to have distinguished three types, which were termed A, B and C respectively.

The more recent researches of the British Committee have shown that all outbreaks cannot be arranged under these heads. The strains examined by Trautwein were collected from outbreaks in Germany, Italy, Jugoslavia, France and England, and the more recent researches of the British Committee indicate that he underestimated the number of distinct types.

Trautwein's classification was based on a comparison with three standard strains selected for the purpose, and he used large numbers of cattle, swine and guinea-pigs.

In 1930, Trautwein and Rappin recorded the investigation at Riems of 176 strains collected from twelve European countries, and found that 144 belonged to type O, 10 to A and 22 to C, while three strains from Switzerland gave irregular results. In 1930, the confidence previously felt by workers in Germany in the comprehensiveness of the immunity produced by the three standard types was shaken by the frequent occurrence of disease in herds which had previously been attacked, where the type of virus appeared at first to be the same in both outbreaks.

Formerly, in outbreaks occurring in Great Britain, only slight evidence of the plurality of types was discovered, but during the last three or four years a number of fresh viruses examined appeared to differ in some respects from all the previously recognised types. Viruses of this sort were isolated during an outbreak imported from Ireland, and another special strain was obtained from an outbreak in Southern Rhodesia. In Southern Rhodesia the outbreaks had shown peculiar features which threw doubt on the diagnosis, the clinical course being very mild and the infectivity very low, with the result that many animals exposed to infection escaped and the disease spread slowly. Attempts to transmit the disease by artificial infection were often unsuccessful. It appeared, however, that the peculiar features displayed by this virus must have been due to local conditions, for the virus, when tested on British cattle at the Experimental Station at Pirbright, set up a severe disease which differed in no way from that produced by English strains.

The cause of these differences between types and strains has not yet been discovered, but it has been recognised that repeated passages of a strain through one species of animal may cause considerable changes in its properties, both in the direction of increasing its virulence for the species and also in altering its resemblance to one or other type.

It now appears that not only is the number of types larger than was formerly believed, but intermediate and even unstable forms occur, though many strains undoubtedly belong to one or other of the sharply-defined types.

It is possible that a real mixture of strains of virus may sometimes occur in outbreaks due to the infection of cattle with two different viruses at the same time, but in experiments carried out by the Committee when an animal was simultaneously inoculated with two strains one usually disappeared while the other persisted.

In the opinion of the Committee, the most probable interpretation of the mixed character of most ill-defined strains is that their components (or antigens) are the same for the most part, but vary in their proportions. Some strains which have defined and relatively stable composition constitute the standard type, but the others are more variable, and at one time or in one animal have one component present in preponderating amount, and on other occasions develop other parts of their structure in greater degree.

The extent to which two or three strains can be used to give immunity against a group of types and their variants is now being tested at Pirbright.

The duration of immunity in cattle and swine which results from an attack of the disease has been tested at Pirbright on a considerable number of animals by exposing them in various ways to a second infection with the same type of virus with which they were originally infected.

In all the recovered cattle which it was possible to test the resistance to infection was found to be very strong, but in swine much less immunity resulted. It was never found possible to overcome the resistance of a recovered bovine animal by intramuscular inoculation or by exposure to contact with an infected animal. When the test was made by direct inoculation into the mucous membrane of the mouth a vesicle was sometimes formed locally and very rarely elsewhere. The resistance to intramuscular inoculation after a single attack and no further exposure was found to be undiminished after 32 months, which was the longest period after which tests were made, or after 52 months since the first infection in the case of cattle exposed to repeated chances of re-infection.

The results in respect of the persistence of immunity were not so satisfactory in the case of swine. At 10 to 11 months after the attack, half of the pigs inoculated became re-infected with generalised lesions by whatever route the virus was introduced.

In order to form an opinion as to the efficacy of serum available from abroad as a preventive against infection, some experiments were made with hyperimmune polyvalent serum made in Germany, which is obtained from cattle that have been subjected to three successive attacks of foot-and-mouth disease with the three main types of virus in succession and afterwards given further inoculations of virus. It is stated by the makers that the serum is active in neutralising the three standard types and it is probably the best procurable.

The results of the experiments carried out by the Research Committee showed that the serum, even in doses larger than those advised by the makers, did not protect cattle or pigs artificially infected or when exposed for 24 hours to contact with an infective animal, but some escaped infection if only exposed for three hours. This was in agreement with experience obtained elsewhere that serum does not protect against free and intimate contact with a diseased animal but only against slight or indirect exposure.

When tested by experiments with guinea-pigs, the serum gave little or no protection against the standard types O and C, but rather better results against the standard type A, against two British strains which did not agree well with any of the standards, and against certain strains from Southern Rhodesia.

The results suggested that not only was the serum examined of low potency but also that it would not have any influence on infections with many of the strains met with in this country.

The question of the relation of the nutrition of animals to their susceptibility to foot-and-mouth disease and to the severity of the disease was

investigated on small species of laboratory animals by Dr. J. T. Edwards. Using chiefly rats and guinea-pigs he found that the factors of age, diet or a concurrent infection influenced the susceptibility to inoculation. It was found that rats and guinea-pigs were most susceptible to foot-and-mouth disease when in middle life, in first rate condition and growing rapidly.

Dr. Edwards suggested that a nutritional factor is also concerned in the annual fall and rise in the incidence of foot-and-mouth disease in India, and that these are related to the seasonal effects of the hot weather and rainfall on the herbage and fodder for the cattle.

The exeprience gained in recent years has again shown that an outbreak of foot-and-mouth disease is occasionally confined to one species of animal. This is in accordance with the experimental evidence that the virus may be adapted to one kind of animal by inoculation of a series of the same species, and in many instances can then only with difficulty be made to infect other species. It is now well-known that a strain of virus straight from cattle is often only adapted slowly to the guinea-pig, while most strains readily infect both cattle and pigs on farms or experimentally. Three strains of virus were specially adapted to swine, but were with difficulty transmitted to cattle. Swine were found to be very resistant to one of the strains obtained from Southern Rhodesia and that was in agreement with the fact that no cases in swine were reported from that country. One strain from an outbreak in cattle in England did not infect pigs exposed to infection in the field and could not be transmitted to swine by inoculation. Extensive outbreaks confined to swine have often been observed in Germany.

With regard to the possible occurrence of persistent carriers of the virus of foot-and-mouth disease among farm animals, the Report observes that such cases can scarcely occur in this country since after the detection of a case of the disease all the implicated cattle, whether actually or potentially infected, are slaughtered.

Further observations were made at Pirbright on a few cattle to test the varied statements made by other workers about the stage of the disease at which the blood, milk and urine are infective. These showed, as had been found by previous experiments, that the virus may be present in considerable concentration in the blood of cattle some hours before there is a rise of temperature or any local lesion, and continues to be found as a rule throughout the period of the formation of vesicles.

In an experiment in which frequent observations were made on three cows, the blood of two of them was infective 3 and 5 hours respectively before vesicles formed or the temperature rose. The appearance of virus in the milk was independent of the time of the infection of the blood; it was never present during the incubation of the disease and was irregular during vesication. There was no relation between the presence of virus in the blood or milk and in the urine. In one of the three cows it was found only in the urine at the 63rd and 89th hour after inoculation; and in another only at the 165th hour three days after the milk and blood had become free, and in the third animal not at all.

The Report points out that in this country the risk of the disease being spread by infected milk or urine comes to an end as soon as a case of disease has been discovered.

In the opinion of the Committee infection is not always transmitted by the same method. Direct contact with contaminated materials has been proved to be efficient in some circumstances, but experiments made by Lebailly and repeated at Pirbright have shown that susceptible cattle as a

rule escape infection when introduced into stalls vacated three or four days before by highly infected bovines. Magnusson in Sweden also found that in such circumstances spread of infection was uncommon and was most likely to occur in cold weather.

The experiments carried out by the Committee did not support the view that cattle were highly infective during the very early stages of the disease before the vesicles had developed. It is thought probable that transmission most readily occurs when a bovine with recently broken vesicles in the mouth and other air passages is near enough to other cattle to infect the air breathed with a spray of minute droplets of buccal or nasal secretion. Work carried out by Dr. Edwards at Pirbright indicated that this route of infection is dangerous for some kinds of animals but not for others. He showed that hedgehogs can give out and take in virus with their breath and become infected in this way, but that guinea-pigs do not give out virus or acquire infection in breathing, and this observation explains the readiness with which the disease spreads among hedgehogs but not from one guinea-pig to another.

Experiments by Dr. Edwards also showed that the disease is more severe and the virus more easily detected in full-grown, well-fed rats, but that in rats on a relatively poor diet inoculation is often ineffectual or the disease remains latent.

Out of 162 wild rats caught in and around the compound at Pirbright, 123 were examined by the inoculation of guinea-pigs, and of these two were shown to be infected in 1932. A rat-proof fence was afterwards erected around the compound and proved to be satisfactory.

In the period covered by the present Report, no infected rats have been discovered on farms during outbreaks of the disease, since the two or three cases mentioned in the last Progress Report.

Field voles have been shown by Dr. Edwards to be readily infected by inoculation. The disease was not severe but vesicles appeared on the tongue.

A grey squirrel and a Syrian hamster were also infected by inoculation by Mr. Galloway.

During 1933, a hedgehog subsequently proved to be infected with foot-and-mouth disease was found and killed on an infected farm where slaughter of cattle had just been carried out, and the dead body was sent to Pirbright. Although no signs of disease were found on the body, foot-and-mouth disease virus was detected in the skin of the feet, in the tongue, and also in the blood and internal organs by the inoculation of guinea-pigs and calves. Fresh, healthy hedgehogs were placed in contact with affected cattle and after four to five days they were found dead with the virus present in their blood. Further, a healthy cow placed in a stall with four infected hedgehogs was attacked with typical foot-and-mouth disease.

The virus recovered from the first hedgehog was of the same type as that obtained from infected cattle on the farm from which it was sent, and it, therefore, seemed probable that this hedgehog acquired the disease from the cattle.

The occurrence of foot-and-mouth disease in a wild hedgehog has, therefore, been proved as well as the possibility of natural transmission from hedgehog to cattle and *vice versa*. No evidence of a similar occurrence had previously been reported.

Mr. Galloway has found that both dogs and cats can be infected by inoculation and also that the disease may pass from one animal to another

of the same species by contact infection. He showed that the three chief different types of virus all caused infection in the cat, and one cat was infected twice, using a different virus on each occasion. These results agree with those obtained by Höve at Riems.

Mr. Galloway, after several unsuccessful attempts to infect ducks by inoculation of the web of the foot, found that if the virus was injected into the thickened pads of skin under the toes infection resulted and vesicles appeared on the upper surfaces of the web. The virus was also present in the blood in some cases as late as the third day after inoculation. No spread of the disease to other ducks by contact occurred.

The Committee think that while these experiments afford additional evidence that some degree of susceptibility is very widespread in the animal kingdom they give no very striking support to the hypothesis of the spread of the disease by birds.

Experiments indicated that the susceptibility of seagulls was not very high. The possibility that they may become infected naturally is not excluded and their possible agency in occasionally transporting the virus mechanically cannot be entirely disregarded.

Since some species of ticks feed on a great many species of animals, it was considered worth while to give attention to the survival of the virus in ticks and the possibility of its transmission by them. Numerous attempts were made by Mr. Galloway to infect ticks from guinea-pigs and to pass on the disease to other guinea-pigs by this means, but transmission by their bites was not effected although it was found that the virus might remain active in ticks for 14 days.

The Report refers to methods of dealing with foot-and-mouth disease abroad and especially to the method of *ring-impfung* systematically pursued in Germany. This consists in isolating the farms involved and giving to all contact animals an inoculation of living virus simultaneously with an injection of the polyvalent hyperimmune serum manufactured at Riems. By this means the immediate contacts are rapidly passed through a mild attack which gives them a more or less lasting immunity, and at the same time animals occupying an area round the zones of infection are protected temporarily by a serum injection which is said to last about 10 days. The serum may, however, mask a mild attack and cases of infection may in this way escape detection. The whole area under treatment is isolated for two to three weeks.

It is recognised that an inoculation of such serum as is at present available cannot be relied upon to protect a bovine animal from infection when intimately exposed to a highly infective case of disease, and experience at Pirbright bears this out.

In Germany, when cattle are sent to shows or markets, they are given a dose of protective serum to diminish the risks of contact with unsuspected cases.

The method has, on the whole, been found satisfactory under the conditions obtaining in Germany. In that country in the years 1921 to 1928, the numbers of cases varied from 11,375 to 12,014, but in 1929 there were only 551 infected farms. It is obviously the great expense that prevents the adoption of the stamping-out method practised in Great Britain. That policy is strictly carried out in Switzerland and the authorities in that country believe that there is good evidence from field observations that recovered cattle can act as carriers and bring about a return of outbreaks.

A NEW DIRECTION IN THE STRUGGLE AGAINST CONTAGIOUS ABORTION.*

The following are the essential points in a method of dealing with contagious abortion recently introduced in Switzerland.

The measures prescribed are not compulsory, that is, not to be enforced by legal measures. Contagious abortion is not included in the list of contagious diseases that owners must notify.

The struggle against the disease is on a voluntary basis, similar to that which is in force in a number of cantons in connection with tuberculosis. Owners who voluntarily come under the scheme must agree to conform to certain prescribed regulations for a period of three years. In return the State will, without any charge, assist in the diagnosis of the disease where that becomes necessary, and they will receive in due course an official certificate certifying that the herd in question is free from contagious abortion.

Detailed explanations will be given to the veterinary surgeons, who will be expected by instruction and advice to stimulate the owners of animals to conform to the method of prophylaxis and in every way to advise and help them. Whereas previously, after diagnosis, the principal procedure was the inoculation of the animals with living or dead cultures, that will cease in the case of herds that come under the new scheme, and in the other herds the vaccination will not be employed except in order to avoid the economic loss in herds with at least 70 per cent. of the animals infected. This measure is intended to prevent vaccinations from being too lightly undertaken and to prevent the creation of excretors of the bacilli.

The new method is based on diagnosis followed by the separation of the infected from the sound animals and the elimination of excretors of the bacilli and of those in which the disease is regarded as specially virulent.

The diagnosis is founded partly on the characteristic clinical symptoms and gross pathological alterations, as well as on the direct or indirect detection of the causal organism.

The principal morbid conditions caused by the abortion bacillus are endometritis and placentitis, which, however, cannot or only with difficulty, be immediately detected and are only recognised by their most important consequence, namely, abortion. That, however, occurs only in about a third of the cases, at the outside in 25 to 80 per cent. of the infected animals, according to the virulence, more frequently in recently infected than in cases of older infection and as a rule are late abortions. These can almost with certainty be regarded as infected with contagious abortion. As a rule, every abortion may be regarded as contagious until some other non-infectious cause is discovered. The infection in the other two-thirds of the infected will be detected in the indirect way through the recognition of specific agglutinin in the blood (or milk) serum.

In all countries serum diagnosis of the disease plays the greatest part, ash as recently been emphasised by the reports of the International Contagious Diseases Office (1936), since the inoculation of animals with dead or living cultures gives rise to the production of antibodies and agglutinins, even in the serum of non-infected animals, such inoculations should be omitted in order to enable one to make a clear serological diagnosis of the disease. An animal that has been treated with dead or living culture may wrongly be regarded as infected and subjected to measures of prevention.

**Schweizer Archiv für Tierheilk.* Bd. 78, 1936, p. 291.

SUCCESS IN DEALING WITH CONTAGIOUS ABORTION IN THE UNITED STATES BY TESTING AND ELIMINATION OF REACTORS.*

Success in curbing Bang's disease through systematic testing and disposal of reactors is shown by studies which the United States Department of Agriculture has made of records compiled on the basis of testing in five Middle West States.

The study indicates that the measures now being used by progressive cattle owners co-operating with State and Federal Officials are successful in the majority of cases. The results were obtained from one original test and three re-tests by the agglutination method of 2,801 infected herds in Wisconsin, Ohio, Illinois, Indiana and Michigan. Testing was conducted under the co-operative plan, in which each owner agrees to establish and follow procedures for preventing reinfection of his herd.

Beginning with 100 per cent. of infection, meaning that all herds contained some infected cattle, nearly half the herds (44·2 per cent.) showed no reactors on the first re-test. On the second re-test, the halfway mark was passed by a considerable margin (54·2 per cent.) and on the third re-test more than three-fourths (75·3 per cent.) of the herds were found to be free of any reacting cattle. The 2,801 herds contained altogether 72,448 cattle. In the course of the four tests the percentage of reactors, based on total number of cattle in all the herds at time of testing, decreased from 26·7 per cent. to 2·7 per cent. By additions to herds of heifers and purchased replacements the total number of cattle in the herds was well maintained after the removal of the considerable number of reactors detected by the first test.

Federal veterinary authorities point out that the probable chief causes of herd infection remaining after three re-tests are: Unusually virulent initial infection, carelessness in the purchase of herd replacements, laxness in maintaining sanitation, and unwarranted confidence in the health of cows passing a single negative test, especially when the herd shows a considerable degree of infection.

THE ERADICATION OF TUBERCULOSIS IN THE UNITED STATES.†

By H. R. Smith (*Live Stock Commissioner*).

In 1916, of all cattle slaughtered under federal inspection, 2·3 per cent. showed lesions of tuberculosis on post-mortem examination. It had increased from 0·9 per cent. in 1908, nearly trebling in eight years. A total of 30,720 cattle and 74,109 hogs were condemned as inedible because of tuberculosis that year, the equivalent of a trainload 20 miles long. Since 1916, 3,500,000 reacting breeding and dairy cattle have been removed from the herds of the United States by the tuberculin test. To-day 43 States and 96 per cent. of all counties are accredited as being virtually free from this disease. New York, New Jersey, Maryland and South Dakota will be officially accredited within a year. California is confronted with organised opposition, but that will be overcome. The percentage of reactors is high in California, which will, therefore, require more time.

Testing Breeding Cattle has nearly eliminated Disease in Market Cattle.— During the fiscal year ended June 30th, 1936, only 0·18 per cent. of all cattle

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**Cornell Veterinarian*, vol. xxvi, 1936, p. 357.

†*Jnl. of the Am. Vet. Vet. Med. Assn.*, 1937, vol. xc, p. 363.

slaughtered, exclusive of reactors, showed lesions of tuberculosis and were designated as retained, as compared with 2·3 per cent. in 1916. There were only 4,027 cattle, exclusive of reactors, and 15,195 hogs condemned for tuberculosis in the United States that year—a trainload only three miles long as compared with 20 miles long in 1916.

The decline in cattle condemnations at Chicago is more impressive than for the nation as a whole—from 4·5 per cent. retained for tuberculosis in 1916, to 0·13 per cent. in 1936. A total of 16,496 or 0·85 per cent. of all cattle slaughtered in Chicago during the fiscal year 1916, were condemned for tuberculosis. During the fiscal year ended June 30th, 1936, only 538 or 0·03 per cent. of the total cattle killed that year, exclusive of reactors, were condemned for this disease.

Twenty years ago, the cattle and hogs condemned for tuberculosis in one year at Chicago alone would fill a train ten miles long, whereas during the past fiscal year, all cattle and hogs condemned for this disease would fill a train two-fifths of a mile long. When South Dakota is accredited, which will be soon, the number of cattle condemned for tuberculosis at this market will be almost nil. This nation-wide cattle-testing programme has thus largely removed a waste of great magnitude and a serious handicap to every producer.

One of the great lessons in these statistics is the efficacy of the tuberculin test. It certainly finds the cattle that have this disease even in the most incipient stage.

Decline in Human Death-rate.—The elimination of tuberculosis in cattle has consistently lowered the incidence of this disease in man. Public health records showed a slight increase in the death-rate from non-respiratory tuberculosis from 21 deaths per 100,000 population in 1900, to 22 in 1916. During that period, there was a considerable decline in the death-rate from respiratory tuberculosis as a result of improved medical practice and better sanitation. This should have brought about a decline also in the death-rate from other forms, including those of bovine origin such as glandular, bone and abdominal tuberculosis, but it was offset by the increase in the prevalence of tuberculosis in cattle during that period. Since the beginning of the federal-State co-operative cattle testing programme in 1917, the human death-rate from non-respiratory tuberculosis has declined to 5 per 100,000 population.

Large Areas now entirely Free.—The fact that there are now a large number of counties in which not a single reactor was found on the last complete test leads one to the conclusion that if the testing is continued the entire nation may eventually be virtually free from bovine tuberculosis. Were it not for the fact that little or no tuberculosis is contracted by cattle from people this would not be possible.

There is little probability that live stock owners will refuse to continue the tests for re-accreditation because our cities will refuse to accept milk or milk products from counties that lose the accredited status. When the entire nation is officially accredited, which will be soon, we should have federal legislation to bar from this country the importation of milk and milk products that do not originate from officially tuberculosis-free areas in foreign countries.

As complete eradication is more nearly approached, the necessity of testing all cattle each three-year period will become less. It is apparent that a complete test once in six years is sufficient when less than 0·2 per cent. reacted on the last county-wide test.

Post-mortem Reports Helpful.—If there can be developed some accurate way to check on conditions without the work and expense of applying the tuberculin test to all cattle at relatively short intervals, it should be adopted. The present practice in some States, of identifying with car-tags bearing recorded numbers all cattle tested so that the few remaining cases of tuberculosis can be reported by the federal Meat Inspection Service, is excellent.

Range cattle are already branded for identification. It is estimated that 60 per cent. of all cattle are slaughtered under federal inspection. If nearly all others were slaughtered under City or State inspection, it would seem that some plan could be devised whereby nearly all cattle could be identified at point of slaughter and the origin of those diseased made known.

Avian Tuberculosis.—The number of cattle condemned for tuberculosis in the United States in proportion to kill is now only one-twelfth as large as 20 years ago. The number of hogs condemned is one-fourth as large as then, in proportion to kill. While the decline in the condemnations in hogs could hardly be expected to keep pace with that of cattle, the principal source of generalised cases in hogs, the decline in hog condemnation is so much less that we may conclude that a considerable number of hogs are condemned for the avian type of infection. From the number of heads sterilised and condemned last year, nearly all of which were infected with the avian type, we estimate the loss on heads at 2,500,000 dollars per year at current values. The wide prevalence of this disease in poultry, particularly in the north central States, constitutes a real problem.

THE OCCURRENCE OF ACID-FAST ACTINOMYCETES IN THE SOIL.*

By Ruth E. Gordon and W. A. Hagan.

A small minority of the actinomycetes are acid-fast. The degree of acid-fastness, however, varies greatly from forms that are almost as acid-fast as tubercle bacilli to others that are only slightly more resistant to decolorisation than ordinary bacteria. The authors note that it is not uncommon in cultures as well as in tissues to see some elements that are distinctly acid-fast and others that are not, and filaments in which only portions are acid-fast.

Acid-fastness in a filamentous organism was first described by Nocard in 1888 in *Actinomyces farcinica*, the cause of "farcin du bœuf," or bovine farcy. Two years later Eppinger found a similar organism, *Act. asteroides*, in a human infection, and in the present century other cases of a similar kind have been described. In 1925, Sanford and Voelker, who collected reports of human actinomycosis in the United States, found that 10 of the 670 cases reviewed were due to acid-fast species. With few exceptions, in all cultures of acid-fast Actinomycetes of which the authors found a record, only four originated in connection with human or animal infections.

Their attention was first drawn to this group of organisms in the course of a study of the ætiology of lymphangitis in cattle (Hagan, *Cornell Vet.*, 1929). In the course of this work they encountered many acid-fast bacteria and a few acid-fast Actinomycetes.

Three of the cultures were obtained from a collection which had been isolated by C. F. Taylor. Examination of this collection of about 100 strains

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Jnl. of Infect. Dis., vol. lix, 1936, p. 200.

showed that only three of them possessed the acid-fast property. They had been isolated on different occasions from scab lesions on potatoes.

Eight additional strains were isolated from soils by the present authors. The procedure for isolation was as follows. Small amounts of soil were placed in test tubes containing 5 c.c. of Söhngen's medium (Frey and Hagan, *J. Infect. Dis.*, vol. xlix, 1931, p. 497). In each tube a glass rod coated with paraffin was placed. After two weeks' incubation at 37° C., the growth on the paraffined rod at the surface of the medium was scraped off and plated on the synthetic medium of Dorset (*J. Am. Vet. M.A.*, vol. lxx, 1926, p. 373). After a few days' incubation, the Actinomycete colonies were easily recognised by their pinkish colour and conical form. Acid-fast forms were not found in every sample. The eight strains were obtained from a series of 30 samples.

Microscopic Appearances.—The authors were unable to discover any feature of the microscopic picture which would have enabled them to differentiate any of the cultures from the others. Smears prepared in the usual way always showed a diversity of forms ; fragments of mycelium, filaments, bacillary forms varying in length, coccoid bodies, and sometimes spiral elements. Frequently one had the impression that he was dealing with a badly mixed culture. These various forms are derived from fragmenting hyphæ and the formation of the elements could be observed by studying the development of surface colonies under the microscope. The youngest colonies appeared as filaments radiating from a centre. True branching occurred abundantly. In the younger elements there was no evidence of fragmentation but after a few days under pressure the older hyphæ in the centre of the colonies broke up into short elements. In most of the cultures aerial hyphæ appeared after a few days' incubation. When stained with the acid-fast technique as a rule part of the elements are acid-fast and part are not. In some filaments coccoid bodies along its course may be acid-fast while the remaining portions are not.

The gross appearance of the growths varied according to the medium. All the strains grew most luxuriously on mediums containing glycerol. The most brilliant pigments ranging from chalky-white through peach and orange to coral and the greatest development of aerial hyphæ were obtained on glycerol potato medium. The strains studied varied more from one another in the shade of pigment formed than in any other cultural feature.

None of the cultures grew under anærobic conditions. On fluid mediums all formed small surface flakes which finally coalesced to form tough, wrinkled pellicles leaving the remaining fluid entirely clear. None of the cultures hydrolysed cellulose suspended in plain broth, liquefied gelatin, hydrolysed starch, produced indol, or digested either blood serum or egg medium. Although the cultures produced heavy pellicles in glucose, lactose and sucrose broths, no gas was produced and at the end of four weeks all tubes were alkaline with a pH of 8·4 to 8·6. All cultures produced acid in glycerol broth and all reduced nitrate to nitrite. At the end of four days, litmus milk cultures were slightly alkaline, at the end of eight weeks they were exceedingly alkaline. There was no reduction of litmus except by *Act. gypsoides* which reduced it slightly and by culture 616 which completely reduced it. That culture also completely peptonised milk. Darkening of the medium containing peptone or meat extract was very striking in plain broth, plain agar, and gelatin inoculated with *Act. gypsoides* and with 616. This coloration of the medium is said to be characteristic of many saprophytic Actinomycetes, the lumpy-jaw organism and a few pathogenic acid-fast Actinomycetes.

Pathogenicity.—The authors' principal interest in the acid-fast Actinomycetes from saprophytic sources was to learn whether or not they possessed pathogenic potentialities. In as much as infections of man and animals with organisms of this group occur so seldom and sporadically, it seemed reasonable to think that the natural habitat of these forms might be in the soil from whence they were carried accidentally into living tissue. For comparative purposes the authors included their strains of *Act. asteroides* and *Act. gypsoides* in the animal inoculation experiments. Rabbits and guinea-pigs were the only experimental animals used. The rabbits were given intravenous injections and the guinea-pigs were inoculated intraperitoneally. If at the end of three weeks the animals were still alive they were killed for examination.

Act. asteroides (401).—This culture proved to be pathogenic for both rabbits and guinea-pigs. A rabbit inoculated with the culture died in a state of emaciation on the 11th day. Autopsy revealed multiple necrotic lesions, 2 to 3 mm. in diameter in the subcutaneous tissues, heart, lungs, liver, spleen, kidneys, stomach and intestinal walls and beneath the peritoneum and pleura. Branching acid-fast elements were readily found in a smear preparation from one of the lesions and the organism was recovered in culture.

A guinea-pig inoculated with this culture died on the 6th day. The autopsy revealed minute flat yellow nodules on the peritoneal surfaces of the body wall, spleen, liver and mesentery, and minute nodules in the lungs and heart muscles. The spleen was adherent to the body wall and a mass of caseous material was found in the folds of the omentum. Numerous beaded, acid-fast elements were seen in a smear of the peritoneal exudate, and the organism was readily recovered in culture.

Act. gypsoides.—This organism was very pathogenic both for rabbits and guinea-pigs at the time of its isolation (Henrici and Gardner), but apparently it had lost its effect upon rabbits, but not for guinea-pigs. An inoculated guinea-pig died four days after inoculation with a heavy suspension. The peritoneum was covered with a diffuse mass of fibrin and many flat nodules had formed on the surfaces of the organs. A single small nodule was found in the lung. Acid-fast filaments were seen in smears from a peritoneal abscess and the organism was recovered in culture.

Culture 568.—This organism was isolated from a potato scab lesion. It was the only one of the " saprophytic " cultures that showed pathogenicity for animals. The culture proved to be highly pathogenic for rabbits but was innocuous for guinea-pigs. Two rabbits inoculated at different times soon after the culture was obtained died in five and thirteen days respectively after the time of injection, about 3 months later a third rabbit was inoculated and it was killed at the end of four weeks. The lesions in the two animals that died were similar to those described for *Act. asteroides.* Thousands of necrotic lesions from 1 to 2 mm. in diameter were found in the lungs, liver, spleen, kidneys, skeletal muscles and heart muscles, and beneath the peritoneal surfaces of the stomach and intestines. Typical branching acid-fast elements were readily found in smears and cultures were easily recovered. The rabbit inoculated three months after the two described and which failed to succumb to the infection, showed about 25 pearly nodules beneath the pleural surfaces of the lungs. Acid-fast elements could not be obtained in smears from these lesions and cultures were not recovered. This animal was certainly recovering from the infection.

Of the remaining ten cultures, nine produced no trace of infection in

rabbits. Strain 632 produced a single nodule about 1 mm. in diameter in the lungs. Acid-fast elements were demonstrated in smears from this lesion and a culture was obtained.

One culture only produced lesions in the guinea-pig. Culture 616 produced abscessation of the omentum. The smears were positive and a culture was obtained.

In conclusion, the authors observe that though the proportion of Actinomycetes that are acid-fast is undoubtedly small, it is obvious that such organisms are not rare in soil. A comparison of the cultural features of these organisms with one another and with those of Actinomycetes isolated from lesions in man and animals shows that a close relationship exists between them. The authors, in fact, had not found any features that would serve to distinguish the " pathogenic " types from the soil types. The character of the pigment formed would distinguish some strains from others, but both pathogenic and soil types are usually pigmented, the colours ranging from yellow through orange to coral. One of the soil types which was highly virulent for rabbits soon after it was isolated produced in that species a disease which was not distinguishable from that produced by the " pathogenic " types. The soil types possess little or no pathogenicity for guinea-pigs, whereas " pathogenic " types produced fatal lesions.

EPIZOOTIC ABORTION OF MARES.*

By W. W. Dimock and P. R. Edwards.

Cases of abortion among mares occur in all parts of the United States. The report deals principally with that form of abortion in mares which has been tentatively designated epizootic or virus abortion.

W. L. Williams appears to have been the first to observe and report an outbreak of equine abortion in the United States, in 1886, and from his description of the outbreaks and the lesions it appears that he was dealing with what is now known as Salmonella abortion.

In 1890, Smith and Kilborne, working in Pennsylvania, isolated from the vagina of a mare that had aborted a bacillus which was shown to belong to the paratyphoid group, but their experimental inoculations had negative results. In 1893, the same authors reported the isolation of a micro-organism belonging to the paratyphoid group that was unquestionably *Salmonella abortivo-equinus*, and they concluded that this organism was the specific cause of abortion among mares. In 1893, Turner reported on a bacillus which was capable of producing abortion in mares experimentally, and afterwards other workers also reported regarding an organism belonging to the paratyphoid group, but streptococci were encountered in some cases.

Serious outbreaks of abortion among mares occurred in Central Kentucky previous to 1911, although they were not officially reported and it is, therefore, impossible to say whether these early outbreaks were of Salmonella abortion or of virus abortion.

Good first isolated *Salmonella abortivo-equinus* in 1911 and his reports show that he did not encounter any outbreaks of equine abortion from which he failed to isolate that organism.

**Cornell Veterinarian*, vol. xxvi, 1936.

In 1907, a laboratory was equipped at the Kentucky Experiment Station for the study of abortion among cattle and horses. At that time there was still great uncertainty as to the relationship of abortion among cows and mares, and particularly as to the specific cause of abortion in mares.

The exact cause of the early outbreaks of abortion in Kentucky was unknown but they were supposed to be caused by *Salmonella abortivo-equinus* until in 1922, the authors observed an outbreak of abortion in a lot of 20 mares that had been immunised against that organism. Eleven of the mares aborted. The agglutination test for it was negative and careful cultural search of all the aborted fœtuses, afterbirths and uterine secretions of the mares also failed to reveal that organism.

Subsequently at least twelve outbreaks of abortion among mares proved, upon examination, to be bacteriologically negative.

It was found that the disease was transmissible by contact, by ingestion of material from the aborted fœtus and afterbirth, and also through the use of material which was passed through filters impervious to ordinary bacteria. Although not all the experiments carried out gave positive results, abortion was produced in a sufficient number to indicate that the disease was infectious and that the causal agent is a filterable virus.

On the basis of present knowledge, abortion among mares may be grouped under the following four heads :—

(1) Salmonella abortion.
(2) Streptococcic abortion.
(3) Virus or epizootic abortion.
(4) Abortion due to miscellaneous causes.

Salmonella Abortion (*Equine Contagious Abortion*).—This is a specific infectious disease of mares due to *Salmonella abortivo-equinus*. It is of an acute character. All available evidence points to the fact that infection is by ingestion and the period of incubation from 14 to 28 days. There is no reliable evidence that the stallion is ordinarily the spreader of infection. The micro-organism usually disappears from the uterus within 30 days following abortion, and except for inflammation due to the entrance of other bacteria the genital tract returns to normal and in a majority of cases the mare will produce a normal foal the following year.

The diagnosis should be confirmed by isolation of the micro-organism from the aborted fœtus, afterbirth, or uterine discharge. It may also be diagnosed by the agglutination test. The disease may be prevented and the outbreaks put under control by vaccination, using a bacterin made from *Salmonella abortivo-equinus*. The generally recommended procedure is to give each mare three injections with intervals of a week. Most cases of this form of abortion have occurred in the latter half of the period of gestation, and vaccination of the mares within three months of the close of the breeding season will almost invariably protect all the mares on the farm.

Streptococcic Abortion.—About 20 per cent. of the observed cases are of this nature. Apparently infection is by the genital tract and never through ingestion or other channels. It may occur before or at the time of service. Diagnosis is made by isolation of streptococci from the fœtus. The afterbirth is usually very abnormal and the mare usually develops metritis following abortion and does not return to normal without treatment. To prevent this form of abortion, care must be taken that all breeding mares are healthy and observe scrupulous cleanliness at the time of service ; in other words, to practise breeding hygiene in every detail.

Epizootic or Virus Abortion.—This often assumes serious proportions. In the first case observed in 1922, 11 out of 20 mares aborted. Other results were 10 out of 14, 24 out of 32, 11 out of 13, and 20 out of 50. In the more recent outbreaks the percentage of abortion has not been so high, perhaps through a better control of the disease by proper handling and isolation of the mares, and through the use of so-called convalescent serum.

Over 125 fœtuses from typical outbreaks of the disease have been subjected to a thorough bacteriological examination. In over 90 per cent. no bacteria of any kind were found. In the few exceptions in which organisms were recovered from one or more of the organs from the body it was evident that they were secondary. Streptococci were isolated from two fœtuses and in eight foals that lived for a short time *Shigella equirulis* was found, but both streptococci and the organism last mentioned are frequently found in fœtuses and foals as prenatal infections.

Tissue changes may be absent in the aborted fœtus, but they are present in the majority of cases and are quite characteristic. In over one-third of the cases serum is present in the thoracic cavity, though this is not so frequent in abortions occurring in the last month of pregnancy as in those that come between the eighth and tenth month of gestation. Probably the most characteristic lesions are small white degenerated areas in the liver, which is gorged with blood and may be somewhat enlarged. These white areas of degeneration occur just beneath the capsule. The mesenteric and colic lymph glands are frequently congested, and petechial hæmorrhages are usually present in the heart.

If in an outbreak of equine abortion the fœtuses show these lesions, no bacteria can be found in the organs or tissues, and the blood of the mares that abort is negative to the agglutination test for Salmonella infection, the disease is probably epizootic abortion, but the diagnosis must often be made by a process of elimination.

The aborting mares seldom show any premonitory symptoms. Generally the afterbirth is passed immediately and the mare quickly returns to normal.

Transmission Experiments.—It has been found difficult to transmit the disease experimentally, notwithstanding the manner in which it manifests itself in a group of pregnant mares.

Various fœtal fluids were used for the infection of mice but the results were negative.

Attempts to infect non-pregnant rabbits by intraperitoneal, intravenous and intracerebral injection all failed. No attempt was made to produce abortion in rabbits on account of their short period of gestation. Similar experiments with guinea-pigs gave indecisive results.

Three pregnant guinea-pigs were inoculated with filtrate of fœtal fluids diluted with an equal quantity of beef infusion broth with a reaction of pH 7·4 and passed through a Seitz filter. The filtrates were bacteriologically sterile. One of the pigs inoculated with filtrate gave birth to one dead pig 14 days after inoculation; one gave birth to three dead pigs 29 days after inoculation, and the third aborted three immature fœtuses 20 days following injection. All these aborted guinea-pigs were negative on bacteriological examination.

The authors consider these results inconclusive.

In all 22 pregnant mares were used in studying the disease. Nine of these were fed and injected with unfiltered material from aborted fœtuses. The materials used included fœtal membranes, fluid and organs.

Of the nine mares used two aborted, respectively 27 and 28 days after inoculation. The fœtuses were bacteriologically negative and presented tissue changes characteristic of the disease.

Ten pregnant mares were fed and injected with filtrate prepared in the same way as those used in guinea-pigs. Three of the mares aborted, respectively 18, 23 and 34 days after inoculation. The fœtuses aborted 18 and 23 days following inoculation showed the pathological changes characteristic of the disease. The third fœtus had been dead for some time before parturition and the organs were soft and degenerated. The remaining mares gave birth to normal foals.

Three mares were used in experiments on stalled exposure. One mare was placed in the stall of an aborting mare on a farm where the disease was present. She was given food that was not being consumed by the aborting mares and boiled water. After being in the stall 60 days, she gave birth to a normal foal.

A second mare placed in a clean, non-infected stall and given water from the same source as that used by the infected mares gave birth to a normal foal after 90 days.

The third mare was placed in a stall where a mare had aborted and was given the same food and water as the infected mares, and on four occasions fœtal membranes of aborted fœtuses were thrown in the stall and left for two or three hours. After 24 days, the mare aborted a fœtus which on post-mortem examination revealed typical lesions.

All the fœtuses aborted by the experimental mares were bacteriologically negative.

In a number of outbreaks a convalescent serum was used as a preventive.

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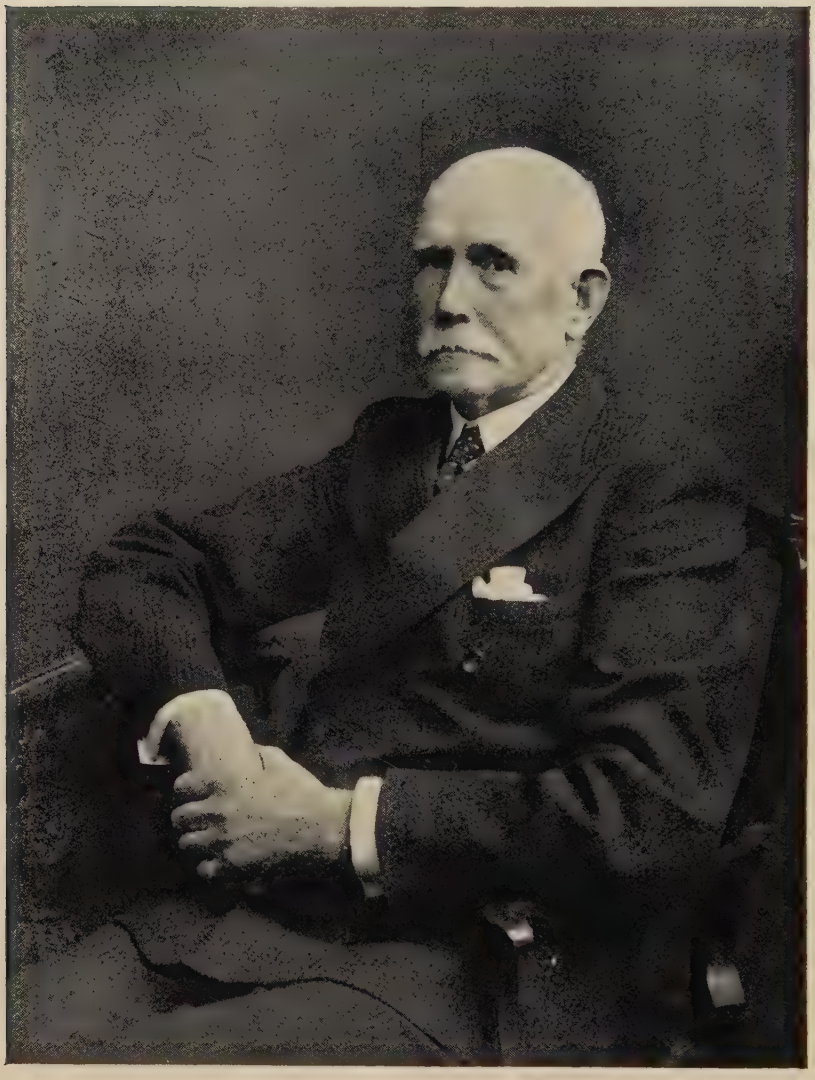

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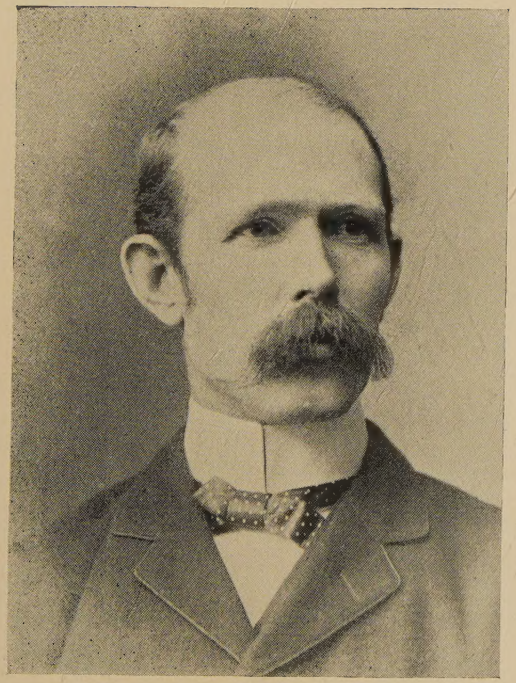

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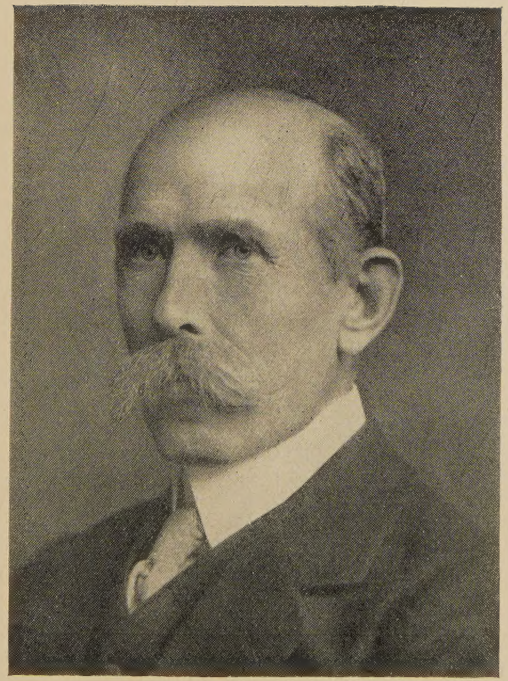
Born June 17th, 1853. M.R.C.V.S., 1876. M.B., C.M., Edinburgh, 1882. B.Sc., Edinburgh, 1883.



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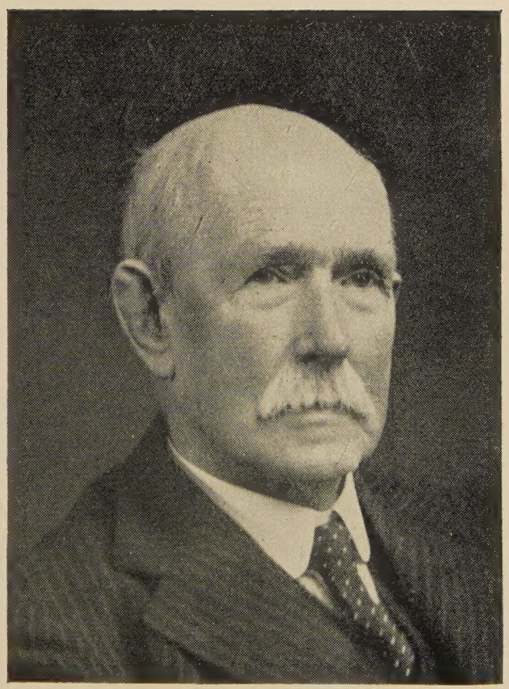
Lecturer in Anatomy, Royal (Dick) Veterinary College, Edinburgh, 1876–1892.



1937

Dean and Professor of Pathology and Bacteriology, Royal Veterinary College, London, 1892–1927. Principal 1894–1927.

EXTRACTS FROM JOHN M'FADYEAN'S INAUGURAL ADDRESSES TO HIS STUDENTS.

1882.

" Constant complaint is heard of that as a profession we are not accorded that place in the public estimation to which we are justly entitled, and many talk as if our position would be made better by Acts of Parliament and things of that nature. The complaint is probably unfounded. It must be confessed that we are yet far from occupying that position in the public regard that it is our ambition to attain to, but it will be wholesome for us to accept the public estimate as the just one, and to set ourselves to find out how our claims to a higher position may be enhanced.

Our title to a higher place must rest on an increased knowledge of the diseases affecting domestic animals, and on an increased power to cure or to prevent those diseases. Will it be believed that there is hardly an important disease peculiar to the domestic animals the pathology of which is fully and accurately known ?

That but little of this work has been done is because the individual members of our profession have not had that scientific training which is necessary to fit them for the task ; and progress will only be made possible by the proper education of the veterinary student. It is the character of the units of the profession that determine the character of the profession as a body. The average veterinary practitioner at any particular period reflects the state of veterinary education at the time when he graduated. The quality and extent of the teaching afforded by the schools is then of paramount importance, and whatever progress we have made, and are still making as a profession, may be traced to the development of veterinary education."

1900.

" . . . There are occupations so mechanical and monotonous as to be little better than treadmills for the grinding out of the necessary portion of daily bread, but the veterinary profession is not one of these. The practice of medicine, whether human or veterinary, presents an endless variety of interesting problems, the solution of which affords an exercise for the most perfectly trained powers of observation and the highest reasoning faculties."

1913.

" . . . And I shall be glad if each of you will consider what I am now going to say in conclusion as addressed to you personally, just as if I had mentioned your name.

" When you pass you will become one of a band of some three or four thousand persons to whom the State has granted certain special privileges. The principal one is the right to call yourself a veterinary surgeon. Let it be one of the chief ambitions of your life to make that designation stand for something higher in the estimation of your fellow citizens.

" With that object you must, in the first place, spare no effort to make yourself master of what is already known regarding the science and practice of your profession. Your efforts in this direction must begin now, and be continued throughout the whole of your professional life. Secondly, you should endeavour to add to the sum of existing veterinary knowledge, so that when your labours are finished your professional brethren may be able to say, " He left our inheritance richer than he found it.' Lastly, but not the least, let your conduct be that of a gentleman in the best sense of that word."

PREFACE.

THE custom of dedicating a *Festschrift* to a distinguished man by his contemporaries, to do honour to him in his lifetime, has not become so well established in this country as it has in Germany. This may be due to differences in standards of taste or merely to timidity. The bestowal of eulogy, it is apprehended, may soon exceed the conventional limits of taste. The processes of criticism, which will be freely employed later by the historian, are perhaps not considered applicable through a certain timidity, and so the high lights of achievement in the lifework of a man cannot properly be thrown up in his lifetime against their less brilliant background. The historian may well find it is in the main the strength of personality of the man—which accordingly escapes critical discussion during his lifetime—that has held his environment in just subduedness to attain along a line of career which he had mapped out for himself some great and beneficial end. It follows that however much his contemporaries acknowledge what may be called the greatness of the man, this quality must be passed by as pervading and indescribable because they commonly feel that it somehow applies to him; but, by them it must not be analysed and described. It must, in any case, be provisional in designation, pending the closer and final scrutiny of the historian. When a man has, however, rendered what it is clearly perceived to be a signal service through his own efforts and initiative, to the lasting good of his fellow men, it would seem that nothing should deter giving expression on fit and proper occasion within his lifetime to the natural, human response of gratitude. Such occasion, it was felt, had arisen at the conclusion of the 50th volume of the *Journal of Comparative Pathology and Therapeutics*, which had been founded in 1888 as a private enterprise by Sir John M'Fadyean, and has since been edited by him almost entirely single-handed. He consented on request to hand over this Number to his colleagues, to prepare with a free hand as a *Festschrift* in his honour. And now it can be declared at once to him that the response to invitations sent to contributors was so prompt, unanimous, and whole-hearted, that no error whatever had been made in discerning the wishes of his many friends and fellow workers throughout the world.

The world which Sir John M'Fadyean for 50 years has addressed in the pages of his journal, therefore, now takes its turn in addressing him. Rejoicing in the knowledge that he keeps good health and that his interest in all the many phases of his lifework remains unabated, those who have felt the pervading and enduring influence of his work —his teachings, writings, counsels, and researches—and, above all, of his example, unite to pay him homage.